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TUESDAY, AUGUST 17, 1976

PART II:

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

■

NEW ANIMAL DRUG APPLICATIONS

Proposals, Withdrawal of Proposals and Opportunities for Hearings

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

[21 CFR Parts 510, 526, 540, 556 and 558]

[Docket Nos. 76N—0173, 0228, 0229, 0230]

FURAZOLIDONE, NIHYDRAZONE, FURALTADONE, NITROFURAZONE

Withdrawal of Proposals and Notice of Proposed Rule Making

Elsewhere in this issue of the FEDERAL REGISTER, the Director of the Bureau of Veterinary Medicine of the Food and Drug Administration (FDA) is issuing notices of opportunity for hearing on proposals to withdraw approval of new animal drug applications (NADA's) Nos. 12–738 and 14–283 (formerly FDC–D–283; now Doc. No. 76N–0229), and NADA No. 65–059 for the use of furaltadone (NF–260); NADA Nos. 13–195 and 13–200 (formerly FDC–D–282; now Doc. No. 76N–0228) for the use of nihydrazone (NF–64); and NADA Nos. 6–395, 6–475, 6–649, 7–801, 8–129, 8–142, 8–324, 8–410, 8–529, 8–784, 9–013, 9–415, 10–741 and 11–154 (formerly FDC–D–280; now Doc. No. 76N–0230), for the use of nitrofurazone (NF–7) in food-producing animals in accordance with section 512(e)(1)(B) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 306b(e)(1)(B)) on the grounds that new evidence not available to him when the applications were approved, evaluated together with the evidence available to him at the time of the approval, shows that those drugs are not shown to be safe for use under either the approved or currently labeled conditions of use. These notices, in addition to that published in the FEDERAL REGISTER of May 13, 1976 (41 FR 19907), regarding furazolidone, constitute due notice that furazolidone, furaltadone, nihydrazone and nitrofurazone are new animal drugs pursuant to section 201(w)(1) of the act (21 U.S.C. 321(w)(1)).

Consistent with this action, the Director is hereby withdrawing the proposal on furaltadone published in the FEDERAL REGISTER of August 4, 1971 (36 FR 14335), and those covering nihydrazone and nitrofurazone published in the FEDERAL REGISTER of March 31, 1971 (36 FR 5918) and is reproposing to revoke the regulations on which these drugs rely as a condition of their approval. Interested persons may submit comments on or before September 16, 1976.

The Director has reviewed the potential environmental impact of the proposal to withdraw approval of NADA's for furaltadone (NF–260); for nitrofurazone (NF–7) and nihydrazone (NF–64) which are subjects of companion notices published elsewhere in this issue of the FEDERAL REGISTER; and for furazolidone (NF–180) which was the subject of notices published in the FEDERAL REGISTER of May 13, 1976 (41 FR 19906 and 19907). He has concluded that the proposed action against these four drugs would not significantly affect the quality of the human environment. An environmental impact analysis report and assessment is on file at the office of the Hearing Clerk, Food and Drug Administration, Rm. 4–65, 5600 Fishers Lane, Rockville, MD 20852.

The Director has carefully considered the inflation impact of the proposed action against the four nitrofuran drugs. Since furazolidone (NF–180) accounts for approximately 97 percent of the usage of nitrofuran compounds in food-producing animals, his review of the inflation impact focused only on the uses of furazolidone. A major inflation impact has been found, as defined in Executive Order 11821, OMB Circular A–107, and the Guidelines issued by the Department of Health, Education, and Welfare. Copies of the inflation impact statement on nitrofurans are on file with the Hearing Clerk, Food and Drug Administration. The major conclusions found in the inflation impact statement are summarized in the May 13, 1976 FEDERAL REGISTER notice concerning furazolidone (NF–180) (41 FR 19906).

Therefore, under the Federal Food, Drug, and Cosmetic Act (sec. 512, 82 Stat. 343–351 (21 U.S.C. 360b)) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.1) and redelegated to the Director of the Bureau of Veterinary Medicine (21 CFR 5.29) (recodification published in the FEDERAL REGISTER of June 15, 1976 (41 FR 24262)), the Director proposes that Parts 510, 526, 540, 556, and 558 of Chapter I of Title 21 of the Code of Federal Regulations be amended, taking into consideration the specific revocations related to furazolidone referred to in the FEDERAL REGISTER of May 13, 1976 (41 FR 19906; formerly FDC–D–281, now Doc. No. 76N–0173), in addition to those relating to furaltadone, nihydrazone, and nitrofurazone, as follows:

§ 510.515 [Amended]

1. Section 510.515 *Animal feeds bearing or containing new animal drugs subject to the provisions of section 512(n) of the act* is amended:

a. By revoking paragraphs (a) (4) and (5);

b. By revoking and reserving paragraph (b) (11) and (15); by redesignating paragraph (b) (17) (i) as (b) (17) and by revoking paragraph (b) (17) (ii);

c. By revoking and reserving in the table in paragraph (c), items 8, 9, and 10.

§ 526.1014 [Revoked]

2. By revoking § 526.1014 *Furaltadone.*

3. By revising § 540.274c(a) (1) and (4), to delete references to furaltadone, as follows:

§ 540.274c Procaine penicillin G in oil.

(a) * * *

(1) *Standards of identity, strength, quality, and purity.* It is exempt from the potency requirement in § 440.274a(a)(1) of this chapter. It is not required to be sterile if it is packaged and labeled solely for udder instillations of cattle. If it is packaged and labeled solely for udder instillations of cattle, the penicillin used is exempt from the requirements of § 40.74a(a)(1) (ii), (iii), and (iv) of this chapter.

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(4) *Requests for certification; samples.* If the batch of procaine penicillin in oil is intended solely for udder instillations of cattle, test results for toxicity, sterility, and pyrogen are not required.

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§ 540.380a [Amended]

4. By deleting from paragraph (a)(1) of § 540.380a *Penicillin ointment* the second sentence, "If it is intended solely for topical veterinary use and not for udder instillation in dairy animals and is conspicuously so labeled, it may contain nitrofurazone."

§ 540.874g [Revoked]

5. By revoking § 540.874g *Procaine penicillin G—furaltadone.*

§ 556.280 [Revoked]

6. By revoking § 556.280 *Furaltadone.*

§ 556.290 [Revoked]

7. By revoking § 556.290 *Furazolidone.*

§ 556.450 [Revoked]

8. By revoking § 556.450 *Nihydrazone.*

9. By amending § 558.15 by deleting references to nitrofuran in the section heading, in paragraph (a), in the introductory text of paragraph (b), and in (b) (1) and (3); by deleting from the table in paragraph (g)(1) the entries for Norwich Pharmacal, and for Hess & Clark and Norwich Pharmacal Co., and by deleting from the table in paragraph (g)(2) the entries for Norwich Pharmacal Co.; as amended ,§ 558.15 (a), (b) introductory text), (b) (1) and (3), and the tables in (g) (1) and (2) read as follows:

§ 558.15 Antibiotic and sulfonamide drugs in the feed of animals.

(a) The Commissioner of Food and Drugs will propose to revoke currently approved subtherapeutic (increased rate of gain, disease prevention, etc.) uses in animal feed of antibiotic and sulfonamide drugs whether granted by approval of new animal drug applications, master files and/or antibiotic or food additive regulations, by no later than April 20, 1975, unless data are submitted which resolve conclusively the issues concerning their safety to man and animals and their effectiveness under specific criteria established by the Food and Drug Administration based on the guidelines included in the report of the FDA task force on the use of antibiotics in animal feeds. All persons or firms previously marketing identical, related, or similar products not the subject of an approved new animal drug application must submit a new animal drug application by July 19, 1973, if marketing is to continue during the interim. New animal drug entities with antibacterial activity not previously marketed, now pending approval or submitted for approval prior to, on, or following the effective date of this publication, shall satisfy such criteria prior to approval.

(b) Any person interested in developing data which will support retaining approval for such uses of such antibiotic and sulfonamide drugs pursant to section 512(l) of the Federal Food, Drug, and Cosmetic Act shall submit to the Commissioner the following:

(1) By July 19, 1973, records and reports of completed, ongoing, or planned studies, including protocols, on the tetracyclines, streptomycin, dihydrostreptomycin, pencillin, and the sulfonamides; for all other antibiotics by October 17, 1973. The Food and Drug Administration encourages sponsors to consult with the Bureau of Veterinary Medicine on protocol design and plans for future studies.

* * * * *

(3) By April 20, 1975, data satisfying all other specified criteria for safety and effectiveness, including the effect on the salmonella reservoir for any antibiotic or sulfonamide drugs approved for subtherapeutic use in animal feeds. Drug efficacy data shall be submitted for any feed-use combination product containing such drug and any feed-use single ingredient antibiotic or sulfonamide not reviewed by the National Academy of Sciences—National Research Council, Drug Efficacy Study covering drugs marketed between 1938 and 1962.

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(g) * * *

(2) * * *

Drug sponsor	Drug premix	Species	Use levels	Indications for use
Commercial Solvents Corp., Thompson-Hayward Chemical Co.	Zinc bacitracin	Chickens, turkeys, swine, pheasants, and quail.	Sec. 558.78	Sec. 558.78.
Do	do	Cattle	Sec. 558.78	Sec. 558.78.
S. B. Penick & Co., Diamond Shamrock Chemical Co.	Bacitracin methylene disalicylate	Chickens, turkeys, and swine.	Sec. 558.76	Sec. 558.76.
Do	do	Cattle	Sec. 558.76	Sec. 558.76.
Do	do	do	Sec. 558.76	Sec. 558.76.
Elanco Products Co	Hygromycin B	Chickens and swine.	Sec. 558.274	Sec. 558.274.
Do	Tylosin	Chickens, swine, and beef cattle.	Sec. 558.625	Sec. 558.625.
Abbott Laboratories	Erythromycin	Chickens, turkeys, and swine.	Sec. 558.248	Sec. 558.248.
The Upjohn Co	Lincomycin	Chickens	Sec. 558.325	Sec. 558.325.
Pfizer, Inc	Oleandomycin	Chickens, turkeys, and swine.	Sec. 558.435	Sec. 558.435.
Hoechst Pharmaceuticals.	Bambermycins	Chickens	Sec. 558.95	Sec. 558.95.
Elanco Products Co	Tylosin	Swine	Sec. 558.630	Sec. 558.630.
Do	Sulfamethazine	do	Sec. 558.630	Sec. 558.630.
American Cyanamid Co., Diamond-Shamrock Chemical Co., Hess & Clark, Rachelle Labs, Inc., and Vitamin Premixers of Omaha.	Chlortetracycline	Chickens, turkeys, swine and cattle.	Sec. 558.128	Sec. 558.128.
Merck Sharp & Dohme Research Labs.	Procaine penicillin.	Chickens, turkeys, swine, pheasant, and quail.	Sec. 558.460	Sec. 558.460.
			Sec. 558.460	Sec. 558.460.
E. R. Squibb & Sons, Inc.	do	do	Sec. 558.460	Sec. 558.460.
Merck Sharp & Dohme Research Labs.	Sulfaquinoxaline	Chickens	Continuously, 0.0125 to 0.025 pct.	Aid in prevention of coccidiosis due to *Eimeria tenella, E. necatrix, E. acervulina, E. brunetti, E. maxima.*
Do	do	Turkeys	Continuously, 0.0175 pct.	Aid in the prevention of coccidiosis due to *Eimeria meleagrides, E. meleagrimites* and *E. adenocides.*
Do	do	Rabbits	Continuously, 0.025 pct.	Aid in prevention of coccidiosis due to *Eimeria stirdae, E. perforans.*
Pfizer, Inc., and Vitamin Premixers of Omaha.	Oxytetracycline	Chickens and turkeys.	Sec. 558.450	Sec. 558.450.
Do	do	Swine (10 to 30 lb).	25 to 50 g/ton	To increase rate of gain and improve feed efficiency.
Do	do	Swine (30 to 200 lb).	7½ to 10 g/ton	Do.
Do	do	Swine	50 g/ton	As an aid in the prevention of bacterial enteritis, also known as scours, baby pig diarrhea, vibrio dysentery, bloody dysentery, and salmonellosis (necro or necrotic enteritis).
Do	do	do	100 g/ton	As an aid in the treatment of bacterial enteritis, also known as scours, baby pig diarrhea, vibrio dysentery, bloody dysentery, and salmonellosis (necro or necrotic enteritis).

Drug sponsor	Drug premix	Species	Use levels	Indications for use
Do	do	do	50 to 150 g/ton	As an idain the maintenance of weight gains and feed consumption in presence of atrophic rhinitis.
Do	do	do	500 g/ton	In presence of porcine leptospirosis reduces instances of abortion, gives higher survival rate, heavier and healthier newborn pigs, reduces urinary shedding of leptospirae and aids in maintenance of normal weight gains and feed consumption. Feed 7 to 14 d, approximately 1 mo before farrowing.
Do	do	Calves	0.05 to 0.1 mg/lb of body weight daily or 25 to 75 mg per head daily.	To increase rate of gain and improve feed efficiency.
Do	do	do	0.5 mg/lb of body weight daily or in complete feed at 50 g/ton.	As an aid in the prevention of bacterial diarrhea.
Do	do	do	0.5 to 5.0 mg/lb of body weight daily or complete feed at 50 g/ton.	As an aid in the treatment of bacterial diarrhea.
Do	do	Cattle	75 to 80 mg per head daily.	As an aid in reducing incidence and severity of bloat. As an aid in reducing incidence and severity of liver abscesses (for cattle weighing over 400 lb). To increase rate of gain and improve feed efficiency. As an aid in increasing milk production in lactating dairy cows.
Do	do	do	0.1 to 0.5 mg/lb of body weight daily.	As an aid in the prevention of bacterial diarrhea.
Do	do	do	0.5 to 5.0 mg/lb of body weight daily.	As an aid in the treatment of bacterial diarrhea, also known as scours.
Do	do	do	0.5 to 2.0 g per head daily.	For the prevention and treatment of the early stages of shipping fever complex. Oxytetracycline is effective prophylaxis when fed 3 to 5 d preceding shipment and/or 3 to 5 d following arrival in feedlots. For treatment of shipping fever, these levels should be fed at onset of the disease symptoms until symptoms disappear.
Do	do	Sheep	10 to 20 g/ton	To increase rate of gain and improve feed efficiency during finishing period.
Do	do	do	50 g/ton	As an aid in the prevention of bacterial diarrhea, also known as scours, lamb dysentery, and white scours of lambs.
Do	do	do	100 g/ton	As an aid in the treatment of bacterial diarrhea, also known as scours, lamb dysentery, and shite scours of lambs.
Do	do	do	25 mg per head daily.	As an aid in reduction of losses due to enterotoxemia, also known as overeating disease.
Pfizer, Inc	Penicillin	Chickens, turkeys, and swine.	Secs. 558.460. and 510.515 of this chapter.	Secs. 558.460 and 510.515 of this chapter.
Do	Penicillin and streptomycin.	do	do	Do.
American Cyanamid Co.	Chlortetracycline	Cattle	Sec. 558.128	Sec. 558.128.
Do	Sulfamethazine	do	Sec. 558.128	Sec. 558.128.
Merck Sharp & Dohme Research Labs.	Procaine penicillin and streptomycin sulfate.	Sec. 558.460	Sec. 558.460	Sec. 558.460.
Abbott Laboratories	Erthromycin	Cattle	37 mg per head per day.	Sec. 558.248.
Hoffman-La Roche, Inc.	Sulfadimethoxine and ormetoprim.	Chickens and turkeys.	Sec. 558.575	Sec. 558.575.
Pfizer, Inc	Oxytetracycline and neomycin.	Chickens, turkeys, swine, and calves.	As provided in paragraph (g)(2) of this section.	As provided in paragraph (g)(2) of this section.
American Cyanamid Co. and Rachelle Labs, Inc.	Chlortetracycline, sulfamethazine, and penicillin.	Swine	do	Do.
Diamond Shamrock Chemical Co.	Chlortetracycline, sulfathiazole, and penicillin.	do	do	Do.

Drug sponsor	Drug premix	Species	Use levels	Indications for use
Do	Chlortetracycline	do	10 to 50 g/ton	Enhancement of growth and feed efficiency.
Do	Arsanilic acid	do	0.005 to 0.01 pct	Do.
American Cyanamid Co.	Chlortetracycline and sulfamethazine.	Cattle	Sec. 558.128	Sec. 558.128.
Pfizer, Inc., and Vitamin Premixers of Omaha.	Oxytetracycline	Chickens	50 g/ton	Prevention of diseases from oxytetracycline susceptible organisms during periods of stress. As aid in the prevention of bacterial enteritis and in the control of neomycin-sensitive organisms associated with blue comb (mud fever or nonspecific enteritis).
Do	Neomycin base	do	35 to 140 g/ton	
Do	Oxytetracycline	Chickens (first 2 weeks).	50 to 100 g/ton	Prevention of early chick mortality due to oxytetracycline-susceptible organism. As an aid in the prevention of bacterial enteritis and in the control of neomycin-sensitive organisms associated with blue comb (mud fever or nonspecific enteritis).
Do	Neomycin base	do	35 to 140 g/ton	Do.
Do	Oxytetracycline	Chickens	50 to 100 g/ton	To extend period of high egg production, to improve feed efficiency, to improve egg production and feed efficiency in presence of disease and at time of stress: As an aid in maintaining and improving hatchability where birds are suffering stress from moving, vaccinations, culling, extreme temperature changes, and worming; to improve livability of progeny when losses are due to oxytetracycline-susceptible organisms, to improve egg shell quality, prevention of blue comb (mud fever or nonspecific enteritis). As an aid in the prevention of bacterial enteritis and in the control of neomycin-sensitive organisms associated with blue comb (mud fever or nonspecific enteritis).
Do	Neomycin base	do	35 to 140 g/ton	Do.
Do	Oxytetracycline	do	100 to 200 g/ton	Prevention of complicated chronic respiratory disease (air-sac infection) and control of complicated chronic respiratory disease by lowering mortality and severity during outbreaks. As an aid in the prevention of bacterial enteritis and in the control of neomycin-sensitive organisms associated with blue comb (mud fever or nonspecific enteritis).
Do	Neomycin base	do	35 to 140 g/ton	Do.
Pfizer, Inc	Oxytetracycline	Turkeys	50 g/ton	As an aid in the prevention of disease from oxytetracycline-susceptible organisms during periods of stress. As an aid in the prevention of bacterial enteritis and in the control of neomycin-sensitive organisms associated with blue comb (mud fever or nonspecific enteritis).
Do	Neomycin base	do	35 to 140 g/ton	Do.
Do	Oxytetracycline	do	50 to 100 g/ton	To extend period of high egg production, to improve egg production, to improve feed efficiency, to improve fertility, to improve egg production and feed efficiency in presence of disease and time of stress; as an aid in maintaining and improving hatchability where birds are suffering from stress, exposure moving, vaccination, culling, extreme losses due to oxytetracycline-susceptible organisms, and to improve egg shell quality prevention of hexamitiasis. As an aid in the prevention of bacterial enteritis and in the control of neomycin-sensitive organisms associated with blue comb (mud fever or nonspecific enteritis).

Drug sponsor	Drug premix	Species	Use levels	Indications for use
Do	Neomycin base	do	35 to 140 g/ton	Do.
Do	Oxytetracycline	Turkeys (first 4 weeks).	50 to 100 g/ton	As an aid in the prevention of early poult mortality due to oxytetracycline - susceptible organisms. As an aid in the prevention of bacterial enteritis and in the control of neomycin-sensitive organisms associated with blue comb (mud fever or nonspecific enteritis).
Do	Neomycin base	do	35 to 140 g/ton	Do.
Do	Oxytetracycline	do	100 to 150 g/ton	As an aid in reducing mortality in birds which have suffered an attack of airsacculitis (it is recommended, wherever possible, to feed from time of attack to marketing).
Do	Neomycin base	do	35 to 100 g/ton	Do.
Do	Oxytetracycline	Turkeys	100 to 150 g/ton	As an aid in the prevention of bacterial enteritis and in the control of neomycin-sensitive organisms associated with blue comb (mud fever or nonspecific enteritis).
Do	Neomycin base	do	35 to 100 g/ton	Do.
Do	Oxytetracycline	do	100 to 200 g/ton	Control of blue comb (mud fever or nonspecific enteritis), infectious sinusitis and hexamitiasis, prevention of infectious synovitis. As an aid in the prevention of bacterial enteritis and in the control of neomycin-sensitive organisms associated with blue comb (mud fever or nonspecific enteritis).
Do	Neomycin base	do	35 to 140 g/ton	Do.
Do	Oxytetracycline	do	200 g/ton	Control of infectious synovitis. For the treatment of bacterial enteritis and blue comb (mud fever or nonspecific enteritis).
Do	Neomycin base	do	70 to 140 g/ton	Do.
Pfizer, and Vitamin Premixers of Omaha.	Oxytetracycline	Swine	50 g/ton	As an aid in the prevention of bacterial enteritis (scours), baby pig diarrhea (in baby pigs only), vibrionic dysentery, bloody dysentery and salmonellosis (necro or necrotic enteritis).
Do	Neomycin base	do	35 to 140 g/ton	Do.
Do	Oxytetracycline	do	50 to 150 g/ton	As an aid in the maintenance of weight gains and feed consumption in the presence of atrophic rhinitis. As an aid in the treatment of bacterial enteritis.
Do	Neomycin base	do	70 to 140 g/ton	Do.
Pfizer, Inc	Oxytetracycline	Calves	50 g/ton	As an aid in the prevention of bacterial enteritis (scours).
Do	Neomycin base	do	35 to 140 g/ton	Do.
Do	Oxytetracycline	do	100 g/ton	As an aid in the treatment of bacterial enteritis (scours).
Do	Neomycin base	do	70 to 140 g/ton	Do.
Do	Oxytetracycline	do	8 to 100 mg/gal reconstituted milk replacer.	As an aid in the prevention of bacterial diarrhea (scours).
Do	Neomycin base	do	100 to 200 mg/gal reconstituted milk replacer.	Do.
Do	Oxytetracycline	do	40 to 200 mg/gal reconstituted milk replacer.	Do.
Do	Neomycin base	do	200 to 400 mg/gal reconstituted milk replacer.	Do.
The Upjohn Co	Lincomycin, amprolium, and ethopabate.	Chickens	Secs. 558.58 and 558.325.	Secs. 558.58 and 558.325.
Do	Lincomycin and zoalene.	do	Secs. 558.325 and 558.680.	Secs. 558.325 and 558.680.
Do	Lincomycin, amprolium, ethopabate, and 3-nitro-4-hydroxyphenylasonic acid.	do	Secs. 558.58 and 558.325 and 558.530.	Secs. 558.58 and 558.325 and 558.530.
Do	Lincomycin, monensin, and 3-nitro-4-hydroxyphenylarsonic acid.	do	Secs. 558.325 and 558.355 and 558.530.	Secs. 558.325 and 558.355 and 558.530.
Merck Sharp & Dohme Research Labs. and Pfizer, Inc.	Procaine penicillin.	Chickens and turkeys.	2.4 to 7.5 g/ton	Sec. 558.460.
Do	Streptomycin	do	12.0 to 37.5 g/ton	Sec. 558.460.
Do	Procaine penicillin.	Chickens	3.75 to 7.5 g/ton	Sec. 558.460.
Do	Streptomycin	do	18.75 to 37.5 g/ton	Sec. 558.460.
Do	Procaine penicillin.	do	3.75 to 30 g/ton	Sec. 558.460.
Do	Streptomycin	do	18.75 to 150 g/ton	Sec. 558.460.
Do	Procaine penicillin.	Turkeys	15 to 30 g/ton	Sec. 558.460.
Do	Streptomycin	do	75 to 150 g/ton	Sec. 558.460.
Do	Procaine penicillin.	Chickens	2.4 to 25 g/ton	Sec. 510.515 of this chapter.

Drug sponsor	Drug premix	Species	Use levels	Indications for use
Do	Streptomycin	do	15 to 75 g/ton	Do.
Do	Procaine penicillin.	Swine	1.5 to 7.5 g/ton	Sec. 558.460.
Do	Streptomycin	do	7.5 to 37.5 g/ton	Sec. 558.460.
Do	Procaine penicillin.	do	7.5 to 45 g/ton	Sec. 558.460.
Do	Streptomycin	do	37.5 to 225 g/ton	Sec. 558.460.
Do	Procaine penicillin.	do	5 to 25 g/ton	Sec. 510.515 of this chapter.
Do	Streptomycin	do	15 to 75 g/ton	Do.
Merck Sharp & Dohme Research Labs.	Procaine penicillin.	do	1.5 to 7.5 g/ton	Do.
Do	Streptomycin	do	7.5 to 37.5 g/ton	Do.
Do	Arsanilic acid	do	45 to 90 g/ton	Do.
Do	Nicarbazin	Chickens	0.01 to 0.02 pct	Do.
Do	Procaine penicillin.	do	2.4 to 50 g/ton	Do.
Do	Nicarbazin	do	0.01 to 0.02 pct	Do.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Do.
Do	Nicarbazin	do	0.01 to 0.02 pct	Do.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Do.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.0025 to 0.005 pct	Do.
Do	Nicarbazin	do	0.01 to 0.02 pct	Do.
Do	Procaine penicillin.	do	2.4 to 50 g/ton	Do.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.0025 to 0.025 pct	Do.
Do	Amprolium	Chickens and turkeys.	0.0125 to 0.025 pct	Sec. 558.55
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Sec. 558.55.
Do	Amprolium	Chickens	0.0125 to 0.025 pct	Sec. 558.55.
Do	Ethopabate	do	0.0004 pct	Sec. 558.55.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Sec. 558.55.
Do	Amprolium	do	0.0125 to 0.025 pct	Secs. 558.55 and 558.530.
Do	Ethopabate	do	0.0004 pct	Do.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Do.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.0025 to 0.005 pct	Do.
Do	Amprolium	Chickens and turkeys.	0.004 to 0.025 pct	Sec. 558.55.
Do	Procaine penicillin	do	2.4 to 50 g/ton	Do.
Do	Amprolium	Chickens	0.004 to 0.025 pct	Secs. 558.55 and 558.530.
Do	Procaine penicillin.	do	2.4 to 50 g/ton	Do.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.0025 to 0.005 pct	Do.
Do	Amprolium	do	0.0125 to 0.025 pct	Sec. 558.55.
Do	Ethopabate	do	0.0004 pct	Sec. 558.55.
Do	Procaine penicillin.	do	2.4 to 50 g/ton	Sec. 558.55.
Do	Erythromycin	do	4.6 to 18.5 g/ton	Sec. 558.55.
Do	Amprolium	do	0.0125 to 0.025 pct	Sec. 558.55.
Do	Erythromycin	do	4.6 to 18.5 g/ton	Sec. 558.55.
Do	Amprolium	do	0.0125 to 0.025 pct	Sec. 558.55.
Do	Ethopabate	do	0.0004 pct	Sec. 558.55.
Do	Amprolium	do	0.012 to 0.025 pct	Sec. 558.55.
Do	Arsanilic acid	do	0.01 pct	Sec. 558.55.
Do	Erythromycin	do	4.6 to 18.5 g/ton	Sec. 558.55.
Do	Amprolium	do	0.0125 to 0.025 pct	Sec. 558.55.
Do	Arsanilic acid	do	0.01 pct	Sec. 558.55.
Do	Ethopabate	do	0.0004 pct	Sec. 558.55.
Do	Amprolium	do	0.0125 pct	Sec. 558.55.
Do	Ethopabate	do	0.004 pct	Sec. 558.55.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Sec. 558.55.
Do	Amprolium	do	0.0125 pct	Sec. 558.55.
Do	Ethopabate	do	0.004 pct	Sec. 558.55.
Do	Bacitracin methylene disalicylate.	do	5 to 35 g/ton	Sec. 558.55.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.00375 pct	Sec. 558.55.
Commercial Solvents Corp.	Zinc bacitracin	do	4 to 50 g/ton	Prevention of coccidiosis; Growth promotion and feed efficiency. Sec. 558.78.
Do	Amprolium	do	0.0125 to 0.025 pct	Do.
Do	Ethopabate	do	0.004 pct	Do.

Drug sponsor	Drug premix	Species	Use levels	Indications for use
Do	Zinc bacitracin	do	4 to 50 g/ton	Prevention of coccidiosis. Growth promotion and feed efficiency. Improving pigmentation. Sec. 558.78.
Do	Amprolium	do	0.0125 to 0.025 pct	Do.
Do	Ethopabate	do	0.0004 pct	Do.
Do	3-nitro-4-hydroxy-phenylarsonic acid.	do	0.0025 to 0.005 pct	Do.
Do	Zinc bacitracin	Swine	10 to 50 g/ton	Increased rate of weight gain and improved feed efficiency.
Do	Arsanilic acid	do	0.005 to 0.01 pct	Do.
Merck Sharp & Dohme Research Labs.	Amprolium	do	0.0125 to 0.025 pct	Secs. 558.55 and 558.530.
Do	Ethopabate	do	0.0004 pct	Do.
Do	Procaine penicillin.	do	2.4 to 50 g/ton	Do.
Do	3-nitro-4-hydroxy-phenylarsonic acid.	do	0.0025 to 0.005 pct	Do.
Pfizer, Inc	Penicillin	Chickens and turkeys.	2.4 to 25 g/ton	Sec. 510.515 of this chapter.
Do	Streptomycin	do	15 to 75 g/ton	Do.
Do	Penicillin	Swine	5 to 25 g/ton	Do.
Do	Streptomycin	do	15 to 75 g/ton	Do.
Dow Chemical Co	Zoalene	Chickens	0.0125 pct	Sec. 558.680.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Sec. 558.680.
Do	Zoalene	do	0.0125 pct	Sec. 558.680.
Do	3-nitro-4-hydroxy-phenylarsonic acid.	do	0.005 pct	Sec. 558.680.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Sec. 558.680.
Do	Zoalene	do	0.0125 pct	Sec. 558.680.
Do	Zinc bacitracin	do	4 to 50 g/ton	Sec. 558.680.
Do	Zoalene	do	0.0125 pct	Sec. 558.680.
Do	3-nitro-4-hydroxy-phenylarsonic acid.	do	0.0005 pct	Sec. 558.680.
Do	Zinc bacitracin	do	4 to 50 g/ton	Sec. 558.680.
Do	Zoalene	do	0.0125 pct	Sec. 558.680.
Do	Penicillin	do	2.4 to 50 g/ton	Sec. 558.680.
Do	Zoalene	do	0.0125 pct	Sec. 558.680.
Do	3-nitro-4-hydroxy-phenylarsonic acid.	do	0.005 pct	Sec. 558.680.
Do	Penicillin	do	2.4 to 50 g/ton	Sec. 558.680.
Do	Zoalene	do	0.0125 pct	Sec. 558.680.
Do	Arsanilic acid	do	0.01 pct	Sec. 558.680.
Do	Bacitracin methylene disalicylate or zinc bacitracin.	do	4 to 50 g/ton	Sec. 558.680.
Do	Zoalene	do	0.0125 pct	Sec. 558.680.
Do	Arsanilic acid	do	0.01 pct	Sec. 558.680.
Do	Penicillin	do	2.4 to 50 g/ton	Sec. 558.680.
Do	Zoalene	do	0.004 to 0.0125 pct	Sec. 558.680.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Sec. 558.680.
Do	Zoalene	do	0.004 to 0.0125 pct	Sec. 558.680.
Do	3-nitro-4-hyrdoxy-phenylarsonic acid.	do	0.005 pct	Sec. 558.680.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Sec. 558.680.
Whitmoyer Labs. Inc	Carbarsone and bacitracin.	Turkeys	Sec. 121.310 of this chapter.	Sec. 558.120.

§ 558.262 [Revoked]

10. By revoking § 558.262 *Furazolidone.*

§ 558.368 [Revoked]

11. By revoking § 558.368 *Nihydrazone.*

§ 558.370 [Revoked]

12. By revoking § 558.370. *Nitrofurazone.*

Interested persons may, on or before September 16, 1976, file with the Hearing Clerk, Food and Drug Administration, Rm. 4–65, 5600 Fishers Lane, Rockville, MD 20852, written comments (preferably in quintuplicate and identified with the appropriate Hearing Clerk docket number found in brackets in the heading of this document) regarding this proposal, except that comments pertaining to issues which are the subject of the related notice of opportunity for hearing published elsewhere in this issue of the FEDERAL REGISTER shall be filed in accordance with that notice. Comments may be accompanied by a memorandum or brief in support thereof. Received comments may be seen in the above office during working hours, Monday through Friday. Comments that have been received in response to th enotice published in the FEDERAL REGISTER of May 13, 1976, regarding furazolidone, will be included with those comments received in response to this notice.

Dated: August 9, 1976.

C. D. VAN HOUWELING,
Director, Bureau of Veterinary Medicine.

[FR Doc.76-23620 Filed 8-10-76;11:19 am]

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

[Docket No. 76N-0172]

HESS AND CLARK ET AL.

Furazolidone (NF-180); Opportunity for Hearing on Proposal To Withdraw Approval of Certain New Animal Drug Applications; Corrections

In FR Doc. 76-13969 appearing on page 19907 in the FEDERAL REGISTER of Thursday, May 13, 1976, the following corrections are made:

1. On page 19907 in the second column, the fourth full paragraph is corrected by deleting the last sentence which reads, "Copies of these studies have been placed on file with the Hearing Clerk, Food and Drug Administration." The studies referred to in this sentence are part of a new animal drug application and are therefore confidential under § 514.111 (21 CFR 514.111).

2. On page 19909 in the first column, in paragraph (4), the words "Project No. 375.09E" are corrected to read, "Project No. 475.09E."

3. On page 19920 in the second column, in paragraph 3., "Vol. 16" is corrected to read, "Vol. 13."

4. On page 19918 in the second column, in the second full paragraph, "May 16, 1972" is corrected to read, "May 26, 1972."

Dated: August 9, 1976.

C. D. VAN HOUWELING,
Director, Bureau of Veterinary Medicine.

[FR Doc.76-23619 Filed 8-10-76;11:19 am]

[Docket No. 72N-0231]

HESS & CLARK, ET AL.

Furaltadone (NF-260); Opportunity for Hearing on Proposal To Withdraw Approval of Certain New Animal Drug Applications

The Director of the Bureau of Veterinary Medicine of the Food and Drug Administration (FDA) is issuing a notice of opportunity for hearing on a proposal to withdraw approval of approved new animal drug applications (NADA's) Nos. 12-738, 14-283 and 65-059 for the use of furaltadone (NF-260) in food-producing animals in accordance with section 512(e)(1)(B) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b (e)(1)(B)) (hereinafter, "the act") on the grounds that new evidence not available when the applications were approved, evaluated together with the evidence available at the time of approval, shows that furaltadone is not shown to be safe for use under either the approved or currently labeled conditions of use. Holders of approved NADA's have until September 16, 1976 to submit requests for a hearing in accordance with § 514.200 (21 CFR 514.200).

In FR Doc. 76-23620 appearing elsewhere in this issue of the FEDERAL REGISTER, the Director is issuing a notice of proposed rule making to revoke the appropriate provisions of sections affected by the proposed withdrawal.

Furaltadone (NF-260) is a member of a class of chemicals called "nitrofurans," which have prophylactic and therapeutic uses in food-producing animals as antibacterial and antiprotozoan agents. In addition to furaltadone (NF-260), which is the subject of this notice, there are three other nitrofurans that are used in food-producing animals and are or have been subject to agency action: Nitrofurazone (NF-7) and nihydrazone (NF-64), which are subjects of companion notices in this issue of the FEDERAL REGISTER; and furazolidone (NF-180), which was the subject of notices in the FEDERAL REGISTER of May 13, 1976 (41 FR 19906, 19907). A fifth nitrofuran, furamazone (NF-84), is not at this time the subject of contemplated agency action for withdrawal. Another proposed agency action concern-furaltadone (NF-260) was published in the FEDERAL REGISTER of August 30, 1974 (39 FR 31678).

Furaltadone (NF-260) was approved in 1962 for treating mastitis in dairy cows via infusion into the mammary gland. It is used in products that are marketed in single-dose units containing 500 milligrams of furaltadone and as a combination drug with each single-dose unit containing 50 milligrams of furaltadone and 100,000 units of procaine penicillin G. The approved treatment in lactating cows is the infusion of one squeeze jet in each infected quarter (an individual mammary gland, including the teat) immediately after milking. Medication remains in the quarter until the next milking. Treatment is repeated after each milking up to a limit of three infusions. For dry cows with a history of mastitis, one squeeze jet is infused into each quarter at the end of the last normal lactation. A second infusion may be given after 5 days.

The NADA's for furaltadone alone are approved with a 36-hour withdrawal for milk, which involves discarding the milk for three milkings. The sponsors, Norwich Pharmacal Co., Division of Morton-Norwich Products, Inc., and Hess & Clark, currently a Division of Rhodia, Inc., voluntarily revised the labels in January 1971, to require a 48-hour withdrawal for milk, which involves discarding the milk from four milkings. The furaltadone combination with penicillin carries a 96-hour withdrawal or eight-milkings discard.

The Food and Drug Administration issued a notice of opportunity for hearing, published in the FEDERAL REGISTER of August 4, 1971 (36 FR 14343), proposing to withdraw approval of NADA's for furaltadone (NF-260) on grounds that:

> Information available to the Commissioner establishes that the drugs, when administered to laboratory animals, have been shown to produce tumors. The drugs are, therefore, not considered to be safe for use in the absence of appropriately sensitive methods of analysis to establish their absence in food derived from treated animals.

Similar notices were published for furazolidone in the FEDERAL REGISTER of August 4, 1971 (36 FR 14343) and for nitrofurazone (NF-7) and nihydrazone (NF-64) on March 31, 1971 (36 FR 5926 and 5927). The history leading to the publication of the 1971 notices is discussed in the May 13, 1976 notice for furazolidone (NF-180).

Norwich and Hess & Clark responded to the August 4, 1971 notice concerning furaltadone (NF-260) by filing requests for a hearing. Norwich assured FDA that it had adequate methods of analysis to determine residues and adequate data to show that under the currently labeled conditions of use, the drugs were safe. In addition, Norwich set forth a hypothesis that nitrofurans were not primary tumorigens but stimulated spontaneous tumor growth by disrupting the normal hormone balance.

Norwich has continued to submit data pertaining to the use of furaltadone (NF-260) in food-producing animals. The Food and Drug Administration has determined that new evidence, evaluated together with the evidence available when the approvals were granted, fails to demonstrate that either the approved or currently labeled uses of furaltadone are shown to be safe. The Director is withdrawing the August 4, 1971 notice of opportunity for hearing concerning furaltadone (NF-260), and is issuing this new notice, which presents in detail all of the evidence now available to him and the basis on which he proposes to withdraw approval of all uses of furaltadone in food-producing animals in accordance with section 512(e)(1)(B) of the act.

Section 512(e)(1)(B) of the act requires that FDA withdraw approval of an NADA when "new evidence * * * evaluated together with the evidence available * * * when the application was approved, shows that such drug is not shown to be safe * * *." Under this provision, the new evidence and the evidence submitted at the time the application was approved must be evaluated to determine whether all of the requirements for safety contained in section 512 of the act continue to be fulfilled.

THE DETERMINATION OF "SAFETY" IN SECTION 512

Section 512 of the act requires that the use(s) of a new drug in food-producing animals must be supported by adequate data supplied by the sponsor in applications that include, among other things, demonstration that the drug use will not result in unsafe residues in human food.

Under section 512(b) of the act, whenever a drug use may result in residues of the drug in food, an application must include not only full reports of investigations to show that the use of the drug is safe, but also a description of practicable methods for monitoring food to assure there are no unsafe residues in human food attributable to the drug use, and demonstration that the conditions of use are adequate to assure there are no unsafe residues.

Under section 512(d)(2) of the act, FDA is required, in the evaluation of the supporting safety data, to consider, among other things:

1. The probable consumption of such drug and of any substance formed in or on food because of the use of such drug, i.e., probable human consumption of residues including the parent drug and metabolites.

2. The cumulative effect on man or animal of such drug, taking into account any chemically or pharmacologically related substance, i.e., toxicological effects of the compounds comprising the residues.

3. Safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of such drugs, are appropriate for the use of animal experimentation data, i.e., establishing "safe" levels of residues using appropriate safety factors to extrapolate animal data on cumulative effects to humans.

Under section 512(d)(1)(H) of the act, additional requirements must be met if the drug is a carcinogen.

The determination of safety within the meaning of section 512 of the act for a drug whose use may result in residues in the edible products of the treated animal, whether or not the drug is a carcinogen, requires a multistep process. These steps form an orderly procedure by which information is derived to satisfy each of the three considerations stated above.

Step 1. Assessment of the total residues in edible products and the depletion of those residues with time following cessation of drug treatment in order to determine probable human consumption (section 512(b)(1) and (d)(2)(A) of the act).

Step 2. Assessment of information about the residues to determine the appropriate testing in laboratory animals required to estimate the cumulative effects of human exposure to the residues (section 512 (b)(1) and (d)(2)(A) of the Act).

Step 3. Acquisition of dose-response data in test animals to determine the cumulative effects of dietary exposure to the residue compounds from which the maximum acceptable concentration of residues in food can be established using appropriate safety factors to assure that human exposure to residues in the total diet does not exceed a level deemed safe (section 512 (b)(1) and (d)(2) (B) and (C) of the act). In the case of a carcinogenic drug, a conservative statistical extrapolation of the tumor data is used to estimate a level of residue exposure that could be considered insignificant and poses essentially no risk of cancer and operationally defines "no residue" for purposes of computing the residue assay requirements under section 512(d)(1)(H) of the act.

Step 4. Acquisition of information about the residues to (a) determine the tissue (called the target tissue) from which residues require the longest time to deplete to safe levels, and which, therefore, must be monitored by the regulatory assay, and (b) determine the compound (called the marker) that is to be measured in the regulatory assay (step 5) so that by its measurement there is assurance that no unsafe residues occur from the use of the drug (section 512 (b)(7) of the act).

Step 5. Development of a practicable method of analysis that can measure the marker in the target tissue at a concentration which assures that total residues in the daily diet of humans do not exceed the level established in step 3 as safe (section 512(b)(7) of the act).

Step 6. Demonstration that practical conditions of use can be prescribed on the label of the product to assure there will be no unsafe residues in the food products from treated animals (section 512 (b)(8) and (d)(2)(D) of the act).

Each of the steps involves principles that are essential for a determination of food safety and compliance with the requirements of the act to assure there are no unsafe residues in human food from the animal drug use. Each step builds upon the preceding step. Therefore, failure to accomplish any one of the six steps in whole or in part for any drug ordinarily will preclude a sponsor's fulfilling its obligation under the act to determine probable consumption of residues and cumulative effects of exposure, and to establish safe conditions of use with appropriate regulatory methods of analysis to control residues when deemed necessary.

New evidence concerning furaltadone (NF–260) not available when the applications were approved includes, among other things, a demonstration that furaltadone induces mammary adenocarcinomas in female rats and may be a more potent carcinogen than furazolidone (NF–180). The Director has evaluated all of the data now available, including the new data received since 1962 when NADA's for furaltadone (NF–260) were first approved and the data available to FDA at the time of approval, and has concluded that the approval of NADA's for furaltadone (NF–260) must be withdrawn under section 512(e)(1)(B) of the act because:

1. Adequate investigations have not been provided to show that the uses of furaltadone are safe, as required by section 512(b)(1) of the act. Adequate information has not been provided on the probable consumption of residues, and the cumulative effects of exposure have not been determined by appropriate tests, as required by section 512(d)(2)(A) and (B) of the act. Therefore, "safe" levels of residue cannot be determined using appropriate safety factors, as required by section 512(d)(2) (C) of the act.

2. Adequate, reliable, and practicable methods of analysis are not available for monitoring food to assure there are no unsafe residues in edible tissues and milk that are attributable to the use of furaltadone, as required by section 512 (b)(7) of the act.

3. Practical conditions of use cannot be specified in the labeling to assure there are no unsafe residues attributable to the use of furaltadone, as required by section 512(b)(8) and (d)(2)(D) of the act.

The data on which these conclusions are based are presented in the succeeding sections of this notice. The Director first describes all the relevant new evidence bearing on the determination of safety for the uses of furaltadone. Then he evaluates all the evidence before him using the six-step procedure necessary to satisfy a human safety determination for the use of furaltadone (NF–260) and to meet the considerations required in section 512 of the act.

I. New Evidence

In October 1966 and January 1967, Norwich submitted reports of the effect in rats of feeding diets containing nitrofurans for 45 weeks. These studies included tests on furaltadone (NF–260) and furazolidone (NF–180). In these studies it was shown that both furaltadone (NF–260) and furazolidone (NF–180) induced mammary tumors in female rats; that furaltadone (NF–260) caused an increase in the number of mammary adenocarcinomas (cancers) which developed in female rats compared with control rats maintained on drug-free diets; and that furaltadone (NF–260) may be a more potent tumor-inducer than furazolidone (NF–180).

Subsequently, more extensive, longer term testing of furazolidone (NF–180) was conducted, but further testing of the carcinogenic potential of furaltadone (NF–260) has not been undertaken. The more extensive testing of furazolidone (NF–180), as described in the May 13, 1976 Federal Register, shows that the drug induces cancer in both rats and mice and that the tumors and cancers were not limited to the mammary gland or the endocrine tissues of female rats.

The Director is including in this notice information available on the toxicological propertes of furazolidone (NF–180) as well as furaltadone (NF–260) because of the structural similarities of the drugs and their similar biological properties, which have been observed in the limited comparative data available. The new evidence showing that furazolidone (NF–180) is a carcinogen has a significant bearing on the Director's conclusions that the uses of furaltadone (NF–260) are not shown to be safe.

Although it is appropriate to consider data on furazolidone (NF–180) to judge the adequacy of existing data to support the safe use of furaltadone (NF–260) in food-producing animals, these data are not a substitute for the data on furaltadone (NF–260) that are required by the statute. Basic data are required for each drug, recognizing that slight differences in chemical structure can result in significant differences in metabolism, residues, and toxicity. Although comparative data on the biochemical and toxicological properties of related drugs may be useful and appropriate in making decisions about the degree of testing required for each drug to show there is no probable hazard, these data cannot be used as a substitute for the basic information necessary on each drug to determine the probable exposure to residues and cumulative effects of exposure.

A. *Carcinogenic Potential of Furaltadone.* Norwich submitted data in October

1966 and January 1967 on the cumulative effects in rats of feeding diets containing furaltadone (NF–260) for 45 weeks ("Effects of Long Term Feeding of Various Nitrofurans," October 13, 1966 and January 4, 1967, Project No. 440.07, submitted to FDA as part of a June 2, 1967, Drug Experience Report). These studies included testing in female Sprague-Dawley (Holtzman strain) rats and testing in both male and female Carworth Farms (CFE strain) rats.

1. The Sprague-Dawley female rat study: Thirty-five female Sprague-Dawley (Holtzman strain) rats were fed furaltadone at a level of about 0.15 percent (85 milligrams per kilogram (mg/kg) body weight) in their diet for 45 weeks, followed by maintenance on an unmedicated diet for an additional 8 weeks before sacrifice. A comparable control group of female rats was maintained on a furaltadone-free diet for 53 weeks. At termination of the study the animals were autopsied, and tissues were examined for histopathological lesions.

The most prominent drug-related effect was the induction of tumors in the mammary glands of the female rats. Although rats develop mammary tumors spontaneously as they age, the incidence of spontaneous mammary tumors in rats just over 1 year old, depending upon the strain of rat used, can be quite low. The furaltadone-treated female rats had an increased number of mammary tumors, particularly the number of fibroadenomas, as compared with the control rats. Even more impotrant, the furaltadone-treated female rats had an increased number of mammary adenocarcinomas compared with the control rats.

Norwich reported that no mammory tumors of any type were observed in the 33 control rats examined. It reported a total of 77 mammary tumors in 25 of the 33 furaltadone-treated rats examined for histopathology. Three of the mammary tumors were reported by Norwich as adenocarcinomas. The report did not indicate which rats had multiple tumors or whether the three adenocarcinomas were in three different rats or included any multiple malignancies.

An Ad Hoc Committee was established to review the pathology data on all the nitrofurans tested in the Sprague-Dawley rat study and the Carworth Farms rat study. One member of the Ad Hoc Committee was the director of the Eppley Institute for Research in Cancer, University of Nebraska. He and his associate pathologists reviewed the pathology slides of the mammary tissues from 33 of the furaltadone-treated rats provided by Norwich and submitted a detailed report to FDA. They did not review all of the mammary gland slides from the control rats. Each of the reviewed slides had a rat identification number, and there were several slides for each rat. However, there was no information on the number of tumors in each rat. Therefore, it was not possible, in many cases, for the pathologists to determine which slides represented different tumors in the same rat and which were different sections of a single tumor.

The Eppley pathologists diagnosed mammary adenocarcinoma in 10 of the furaltadone-treated female rats, and found apparent multiple tumors, predominantly fibroadenomas, in 25 of the treated rats. However, because the Norwich report did not indicate which 25 rats had tumors, it is not possible from the data available to determine if the tumors reported by the Eppley pathologists were from the same 25 rats in which Norwich had reported tumors. The Eppley pathologists did report a considerably higher number of rats with mammary adenocarcinoma than was reported by Norwich. However, because the Eppley pathologists did not review all of the mammary gland slides from the control rats, a comparative incidence of mammary cancer cannot be determined from the reports.

A pathologist from the Bureau of Foods of FDA, not a member of the Ad Hoc Committee, later reviewed the pathology slides of the mammary tumors from the 25 furaltadone-treated rats in which the Eppley pathologists had reported mammary tumors. From his examination of the histopathological preparations provided, he reported 4 slides of mammary fibroma from 3 rats, 70 slides of mammary fibroadenoma from 23 rats, and 14 slides of mammary adenocarcinoma from 10 rats. The mammary adenocarcinomas were diagnosed in the same 10 rats in which the Eppley pathologists had diagnosed adenocarcinoma.

The Bureau of Foods pathologist also did not have information indicating which slides were multiple sections of single tumors or which represented different tumors. He did have information on the rat identification number for each set of slides. Because the Bureau of Foods pathologist did not review all of the slides in the study, tumor incidence and the comparison of incidence with the control rats cannot be determined from his report. However, his report supports the Eppley pathologists' findings that mammary tumors were found in at least 25 of the furaltadone-treated rats, and mammary adenocarcinoma was found in at least 10 of the furaltadone-treated rats.

2. The Carworth Farms rat study: Furaltadone (NF–260) was fed to 20 male and 20 female Carworth Farms '(CFE strain) rats at about 0.15 percent in the diet (67 to 84 mg/kg body weight) daily for 45 weeks, followed by maintenance on an unmedicated diet for an additional 7 weeks before sacrifice. A comparable control group of rats was maintained on a furaltadone-free diet for 52 weeks. At termination of the study the animals were autopsied, and the tissues were examined for histopathological lesions.

As in the previous Sprague-Dawley rat study, the most prominent drug-related effect seen was the induction of tumors in the mammary glands of the female rats. This included an increase in the number of mammary fibroadenomas and an increase in the number of mammary adenocarcinomas.

No mammary tumors were found by Norwich in the 15 female control and 18 male control rats examined. Norwich reported that 14 out of the 17 furaltadone-treated female rats examined had a total of 49 mammary tumors. Norwich also reported that five mammary adenocarcinomas were found in the furaltadone-treated female rats but did not report whether these adenocarcinomas were seen in five different rats or whether some of these were multiple tumors in the same rat.

The Eppley pathologists reviewed the slides of the mammary tissues from 17 furaltadone-treated rats provided by Norwich. They concluded that 5 of the furaltadone-treated female rats had adenocarcinoma of the mammary gland and that mammary tumors, primarily fibroadenomas, were present in 14 of the furaltadone-treated rats. Slides of the mammary tumors reported by the Eppley pathologists were later reviewed by the Bureau of Foods pathologist. He reported mammary tumors in each of the 14 rats. From his examination of the histopathological preparations provided, he reported 4 slides of fibroma from 4 rats, 38 slides of fibroadenoma from 8 rats, and 11 slides of adenocarcinoma from the same 5 rats in which the Eppley pathologists had reported adenocarcinoma.

Because Norwich did not report the number of rats in which mammary adenocarcinoma was found, and because the Eppley and Bureau of Foods reviewing pathologists did not review mammary tissue slides from control rats, and because the Bureau of Foods pathologist did not examine all the slides from the treated rats, the incidence of mammary cancer cannot be determined from the available reports. However, the Eppley and Bureau of Foods reviewing pathologists did concur in the finding of mammary adenocarcinoma in at least five furaltadone-treated female rats from the study.

Food and Drug Administration scientists recognize that there are uncertainties and limitations in the examination of tumors to determine if they have malignant potential. For example, in the early stages of a mammary tumor growth, the mass may be chiefly adenomatous. As the growth continues, the supporting white fibrous connective tissue may also begin to proliferate, resulting in a fibroadenoma. Later the adenomatous part of the neoplastic mass may entirely disappear, leaving a fibroma. Finally, a part of the adenoma or fibroadenoma may develop malignant tendencies and that portion may then become an adenocarcinoma. All four tumor types, i.e., adenoma, fibroadenoma, fibroma, and adenocarcinoma may be present in the same tumor mass. A small focus of adenocarcinoma in the early stage of a malignant tumor may be overlooked in the tumor growth unless adequate multiple sections of the tumor are made.

Also, unless great care is exercised in the evaluation of the spread of an adenocarcinoma of the mammary gland, the metastasis along the lymphatic routes passing through, under or near other

mammary glands may be considered to be separate primary adenocarcinomas rather than a single primary growth with multiple metastases.

The reports from Norwich indicate that furaltadone caused an increase of mammary tumors in female rats just over 1 year old and caused an increase in the number of mammary adenocarcinomas compared with control rats.

Food and Drug Administration scientists conclude that both studies show that furaltadone is tumorigenic in rats, and that the data raise questions about the carcinogenic potential and potency of furaltadone. They conclude that neither of the studies is adequate to assess the cumulative effects of exposure to furaltadone and its carcinogenic potential and potency because the studies were not of sufficient duration and the number of animals used was not sufficient. In the Sprague-Dawley rat study only female animals were used, so it is not possible to determine whether furaltadone might have produced effects in the male rats. In the Carworth Farms rat study, the number of males was too small to determine with any confidence that furaltadone did not have an adverse effect. In both studies drug treatment was for only 45 weeks and included only one dose-level. In spite of the weaknesses in the reports available, it is evident that a 45-week treatment was sufficient to induce tumors in female rats, and that some of these tumors were mammary cancers. The dose level, frequency, and duration of exposure to a compound are related not only to whether or not cancer is induced but also to the latency period before the expression of cancer. Therefore, the 45-week treatment with furaltadone is not considered a sufficient period of exposure to determine adequately the cancer potential or potency.

The Director concludes that the available data provide sufficient cause for concern that furaltadone may be a carcinogen and that adequate lifetime testing in appropriate animal species is required to evaluate its carcinogenic in potential and potency. The Director concludes that because furaltadone is a tumorigen, dose-response data in lifetime studies, using several dose levels, are necessary to develop the information required to assess the cumulative affects of exposure and to establish conditions of safe use. Although the demonstration of cancer induction may be made on the basis of testing at only one dose level and it may be possible from the results of this testing to conclude that a drug requires regulation under the provisions of section 512(d) (1) (H) of the act, dose-response data are necessary to provide information from which FDA can establish the operational definition of "no residue" for purposes of determining the required conditions for the regulatory assay for residues of a carcinogenic drug. Regardless of whether adequate testing were to show that furaltadone induced cancer or induced only benign tumors, dose-response information would be required to establish the conditions of safe use.

The Norwich data from these two studies were available at the time FDA issued the August 1971 FEDERAL REGISTER notice of opportunity for hearing on the proposed withdrawal of approvals for furaltadone (NF–260). In response to the August 1971 notice, Norwich stated that all four nitrofurans (NF–260, NF–7, NF–64, and NF–180) caused an early development of mammary tumors that were of the type normally found in aging rats, and it postulated that the nitrofurans caused these tumors only at high levels of exposure in the female rats because of disruption in the hormonal system. Norwich presented data purporting to show that the hormonal disruption resulting in the tumors involved a process not applicable to humans and that the proposed methods of analysis for control of residues were more than adequate to assure that levels of residues were below any level which might adversely affect humans.

The Norwich hormonal hypothesis is described in detail in the May 13, 1976 FEDERAL REGISTER notice on furazolidone (NF–180). The supporting information provided by Norwich for the hypothesis was primarily limited to data on furazolidone (NF–180) and some very limited comparative data on nitrofurazone (NF–7). No data were presented on furaltadone (NF–260) related to a hormonal mechanism of action.

B. *Comparative Data on Furaltadone and Furazolidone.* Norwich has provided data on furazolidone (NF–180) from the Sprague-Dawley female rat study and the Carworth Farms rat study. It has also supplied data on testing of furazolidone (NF–180) in three chronic rat studies and one mouse study; the tests are described in the May 13, 1976 FEDERAL REGISTER notice. The data have a bearing on the Director's proposed action in this notice for furaltadone (NF–260) and his decision to reject the Norwich hormonal hypothesis for furaltadone (NF–260) and furazolidone (NF–180). They also have a bearing on his decision to reject the sponsors' claims that adequate studies have been provided for furaltadone (NF–260) to support a determination that the drug uses are shown to be safe.

In the Sprague-Dawley female rat study and Carworth Farms study, furazolidone (NF–180) was also tested in the protocols using a dietary level of about 0.1 percent as compared to the furaltadone (NF–260) dietary level, which was about 0.15 percent.

In the first study, Norwich reported that 14 of the furazolidone-treated Sprague-Dawley female rats out of 32 rats examined had mammary tumors. The total number of tumors reported by Norwich was roughly half the number of tumors reported for the furaltadone-treated rats. Norwich reported 5 adenocarcinomas in the mammary glands of the furazolidone-treated rats but did not specify in which rats these were found or whether any were multiple malignancies.

The Eppley pathologists reviewed the mammary tissue slides from 32 furazolidone-treated female rats and reported tumors in 14 of the rats. The Bureau of Foods pathologist later reviewed slides of the mammary tumors from the 14 rats and reported tumors in all of these rats. Both the Eppley and Bureau of Foods pathologists reported only one mammary adenocarcinoma and one mammary squamous cell carcinoma, each in a different rat, from the slides of the furazolidone-treated rates they had examined. In contrast to these furazolidone-treated rats, the Eppley and Bureau of Foods pathologists had reported mammary adenocarcinomas in slides from 10 rats treated with furaltadone, indicating a significant increase in the number of mammary adenocarcinomas from the furaltadone-treated rats compared to the furazolidone-treated rats.

In the Carworth Farms rat study, 15 of the furazolidone-treated female rats out of 17 examined were reported by Norwich as having mammary tumors. In comparison, Norwich had reported that 14 of the female rats treated with furaltadone (NF–260) had mammary tumors. The total number of mammary tumors in the furazolidone-treated female rats was greater than the total number of mammary tumors in the furaltadone-treated female rats, but the number of mammary adenocarcinomas and the incidence of rats with adenocarcinomas was greater for the furaltadone-treated female rats compared to the furazolidone-treated female rats. Norwich reported only two mammary adenocarcinomas in the furazolidone-treated female rats, but did not designate whether these were in one or two rats. The Eppley pathologists diagnosed mammary adenocarcinomas in two furazolidone-treated female rats. The Burau of Foods pathologist also reported adenocarcinoma in the same two rats. He further diagnosed tumors from two additional rats as also showing low-grade mammary adenocarcinoma. Tumors from these two additional rats had been diagnosed as fibroadenomas by the Eppley pathologists. In contrast Norwich had reported five adenocarcinomas in the furaltadone-treated rats and the Eppley and Bureau of Foods pathologists diagnosed mammary adenocarcinomas in five of the furaltadone-treated rats, indicating an increase in the number of adenocarcinomas for furaltadone-treated rats compared to furazolidone-treated rats.

Based on these comparative data, the Director concludes that furazolidone (NF–180) and furaltadone (NF–260) qualitatively exhibit very similar tumorigenic effects in female rats. Because the dose levels of the two drugs were not the same (furaltadone (NF–260) at about 0.15 percent in the diet and furazolidone (NF–180) at about 0.1 percent in the diet) and because the studies are not adequate to assess the cumulative effects of exposure and carcinogenic potential and potency, the data are not sufficient for a precise comparison of the tumorigenic potencies. However, based on the limited comparative data available, furaltadone (NF–260) may have a greater carcinogenic potential than furazolidone (NF–180).

More definitive studies of the carcinogenic potential of furazolidone (NF–180) in male and female rats and mice provided by Norwich are described in the May 13, 1976 FEDERAL REGISTER notice. A summary of the findings from the three chronic rat studies and the mouse study include the following:

1. Furazolidone induced malignancies in both rats and mice.

2. Furazolidone also induced nonmalignant tumors.

3. In most cases, the induced malignant and nonmalignant tumors were found at the same sites.

4. There is some evidence that the induction of benign tumors is related to the induction of, or progression to, malignancies as a function of dose.

5. There are marked sex differences in the tumor responses in the rats. There are also some commonalities in the tumor sites for the male and female rats.

6. Mammary tumors were the predominant effect in the female rats.

7. Nonmammary tumors were induced in the Sprague-Dawley male rats, with no one type singularly significant. In the Fischer rats, however, several specific nonmammary tumors were induced in both male and female rats.

8. The major target tumor site in the mouse was the lung. There was no evidence of a significant difference between male and female mice in tumor response, and there were no tumorigenic effects in the endocrine tissues.

9. The induction of tumors involved not only increased incidence of animals with tumors but also an increased number of tumors per animal.

The Director has rejected the data supplied by Norwich to support the hormonal hypothesis for furazolidone (NF–180). A discussion of these data and his basis for rejection are also presented in the May 13, 1976 FEDERAL REGISTER notice. The Director also rejects the Norwich reply to the 1971 FEDERAL REGISTER notice on furaltadone (NF–260) attempting to explain the tumorigenic effects of furaltadone primarily on the basis of the furazolidone (NF–180) data relative to the hormonal hypothesis. The Director finds that the new evidence provided by the more extensive chronic testing data for furazolidone (NF–180), which shows that tumors were induced in both male and female rats and mice and were not limited to tumors of the mammary gland or the endocrine tissues, is inconsistent with the hormonal hypothesis.

Therefore, the Director concludes that the comparative data available on furazolidone (NF–180) and furaltadone (NF–260) in the Sprague-Dawley female rat study and the Carworth Farms rat study, together with the data on the four chronic studies of furazolidone (NF–180) in rats and mice, indicate that adequate testing of furaltadone (NF–260) has not been provided to assess the cumulative effects of exposure necessary for a determination of safety.

II. USES OF FURALTADONE ARE NOT SHOWN TO BE SAFE

To assess the safety of residues using criteria described in section 512(b) of the act and elaborated on in section 512(d)(2) of the act requires a multistep process that must take into account consideration of the metabolism of the drug, determination of the resulting residues, including parent drug and the metabolites, the depletion of those residues with time after drug treatment, and the cumulative effect of human ingestion of the residues. The six basic steps involved in this evaluation process have been previously described in this notice.

A. *The Amount of Total Residue in the Edible Products and the Depletion of Residues with Time Has Not Been Determined (Step 1).* When a drug is administered to an animal, the drug may be altered by the animal into a variety of compounds called metabolites. The process of alteration may be such that little residue of the parent drug remains in the animal tissue, but the amount of metabolites may be substantial. Therefore, the total residue with which FDA is concerned includes both the parent drug and the metabolites.

The nature and amount of each of the residues depend in part upon the metabolism in a particular species. Because there are species differences in metabolism, it is necessary to determine metabolism and residues in each of the species for which the drug is used. Therefore, § 514.1(b)(7)(i) (21 CFR 514.1(b)(7)(i)) includes metabolism and residue depletion studies among those kinds of studies that are used to determine residues.

Exposure to furaltadone in the diet induced mammary tumors and adenocarcinomas in female rats. It is not known whether the parent drug was the active agent or whether the responses were due to one or more active metabolites produced as a result of ingestion of the parent drug by the rats. Such lack of knowledge is not unique to furaltadone; it is typical of many tumorigenic and carcinogenic agents that the mechanism of the induction of tumors or the identification of the active agent is not known. Therefore, when considering the safety of food from furaltadone-treated animals, all drug-related residues to which humans may be exposed must be assumed to be potentially carcinogenic unless convincing evidence is presented to the contrary. Such evidence consists of not only the identification of the residues that are claimed to be safe, but also adequate toxicological studies showing that the levels of each of the identified claimed to be safe are in fact safe. All residues not identified or not shown to be safe must be considered potentially carcinogenic and must be required to be below a level of measurement that can be considered insignificant and to pose essentially no risk of cancer in humans.

The use of chemical assays to determine initially the total residues would require knowledge about what residues occur (parent drug and metabolites) and would require the availability of adequate methods of analysis to measure each one of the residues at the level of interest in the edible tissues. Therefore, this chemical approach is usually not feasible. Instead, a radiolabeled parent drug is used to provide a practical and adequate approach to the initial measurement of total residues in the edible tissues. In this approach, the drug is synthesized with a radioisotope so that when the drug is administered to the animal according to label directions, all resulting residues of potential interest can be measured in the edible products. This is accomplished by counting the radioactivity in the tissues and converting these measurements to an estimation of the amount of residue expressed as parent drug equivalents.

No data have been provided by the sponsors to measure total residues in milk or in edible tissues from furaltadone-treated animals. Similarly no data have been provided to characterize the residues occurring in the edible products. It has been shown that furaltadone is found as a residue in milk following drug treatment, as will be described in paragraph F. (step 6) below in this section, but it has not been shown what proportion of the total residue is the parent drug.

In FDA experience with the use of drugs injected into the mammary gland for treatment of mastitis, it has been shown that such drug use may result in systemic absorption leading to residues in the edible tissues as well as in the milk. No data have been supplied by the sponsors of furaltadone to show whether or not the use of furaltadone results in residues in the edible tissues.

Although no data of any type have been supplied on the metabolism of furaltadone in cows, very limited information in the following references has been provided regarding rats and dogs.

1. Paul, M. F., et al., "Studies on Distribution and Excretion of Certain Nitrofurans," Antibiotics and Chemotherapy, 10:287–302, 1960.

2. Bugard, J. A. and J. D. Conklin, "Distribution of Furaltadone in the Rat," Antibiotics and Chemotherapy, 11:89–96, 1961.

Data from these studies show that in the rat, furaltadone is absorbed from the small intestine and colon, and that in the excreta less than 5 percent of the administered dose is eliminated as the intact parent drug. The in vivo data are limited to the examination of blood and excreta and do not include any structural characterization of metabolites or the examination of tissue storage and distribution. The references also provide the results of some in vitro studies using liver, kidney, and intestinal homogenates prepared from rats and dogs. Furaltadone was added to the homogenates, and it was shown under these conditions that the biological half-life of the parent drug was about 35 minutes in the rat and 37 minutes in the dog. Both rats and dogs appear to rapidly metabolize furaltadone. These data reinforce the Director's conclusion that consideration must be given to metabolism in the animal of the intended drug use to assess the residues and their significance as contaminants in food.

The Director concludes that adequate information on metabolism of furaltadone in the cow and the determination of total drug residues and their depletion in both edible tissues and milk has not been provided. Therefore, the first step necessary to estimate probable consumption of residues, which is a required consideration of section 512(d)(2)(A) of the act, has not been fulfilled.

B. *Sufficient Information Has Not Been Provided about the Residues to Determine the Appropriate Testing Required in Laboratory Animals to Estimate the Cumulative Effects of Human Exposure to the Residues (Step 2).* The composition of a drug's residue may vary from the extremes of less than 1 percent parent drug and more than 99 percent metabolites to less than 1 percent metabolites and more than 99 percent parent drug, though the latter case would be rare. The Food and Drug Administration must assess the toxicity of the total residue because this is what man will be exposed to in food. Ideally, this assessment would be based on the identification of each of the metabolites in the residue, their synthesis, and subsequent testing of the parent drug and all of the metabolites for the cumulative effects of exposure. However, this approach is usually impractical.

Various approaches are available to limit the number of residue compounds for which toxicity testing is required to provide a sufficiently conservative estimate of the toxicity of the total residue. However, Norwich has not presented any quantitative or qualitative information about the total residues of furaltadone in edible tissues or milk.

Therefore, the Director concludes that not only is the toxicological testing provided for furaltadone inadequate, but he is unable to determine whether testing of metabolites in addition to the parent drug is required to determine the safe level of human exposure to residues in food. Step 2 has not been fulfilled; therefore the requirements for step 3 cannot be fully determined.

C. *Adequate Dose-Response Data in Test Animals to Determine the Cumulative Effects of Dietary Exposure to the Residue Compounds Have Not Been Provided; Therefore, the Maximum Acceptable Concentration of Residues in Food Cannot be Established Using Appropriate Safety Factors to Assure that Human Exposure to Residues in the Total Diet Does Not Exceed a Level Deemed Safe (Step 3).* As discussed under section I of this notice, the Director has concluded that adequate testing of the parent drug furaltadone has not been provided. Also, under paragraphs A and B (steps 1 and 2) of this section, the Director concluded that adequate information has not been provided about the residues to determine if toxicity testing of residue compounds in addition to the parent drug is required to estimate conservatively the cumulative effects of exposure to the residues.

The Director concludes that without such essential information about the residues and adequate toxicity testing data, he cannot, in light of the new evidence, determine if furaltadone requires regulation as a carcinogenic animal drug nor can he reasonably estimate a maximum acceptable level of residue in food that can be deemed safe. Therefore, he concludes that adequate information has not been provided to assess the cumulative effects of exposure and to determine the appropriate safety factors required in section 512(d)(2)(B) and (C) of the act.

D. *Sufficient Information about the Residues Has Not Been Provided to Establish the Conditions Necessary for the Analytical Method Used to Control Residues (Step 4).* The evaluation of an analytical method for residues first requires a determination of the tissues to be analyzed, the compound to be measured, and the lower limit of measurement that must be achieved to control total residues adequately. Only then, after selection of these parameters, is it meaningful to consider whether the method proposed by the sponsor meets other characteristics necessary for a practicable reliable method.

1. Determination of the target tissue: A probability exists that residues of furaltadone occur not only in milk, which must be monitored, but may also occur in edible carcases tissues. The most practical approach to monitoring and controlling residues in the field is dependent on the selection of that edible tissue in which residues require the longest time to deplete to a "safe" concentration." Therefore, by monitoring this tissue to assure that residues are below a "safe concentration," it can be assured that all residues in the carcass are below "safe concentrations." In lieu of this determination (identification of the target tissue), all edible tissues, including milk where applicable, must be monitored. This would place an additional burden on public funds for monitoring.

The target tissue is readily identified if the requirements of step 1 of the evaluation process are met: either by showing that there is no systemic absorption of the drug from the udder that causes residues in tissues at any time after drug treatment, or by showing that if tissue residues occur, from what edible tissues the total residues require the longest time to deplete. Norwich has failed to meet these requirements. Therefore, it cannot be determined if analytical methods are required for tissues in addition to methods of analysis for milk.

2. Determination of the compound (marker) which can be measured by the analytical method to assure control of total residues: Because the radiolabeled drug approach used initially to estimate total residues is not applicable to regulatory use, an alternative approach must be developed to assure control of total residues after drug approval. This is achieved by selecting an appropriate residue compound, which may be the parent drug or one or more metabolites, in such a way that its measurement serves as a valid indicator that when the marker compound is below a certain level in the target tissue, all residues in edible tissues are at safe levels. This step is essential to determine if the method of analysis for regulatory use is adequate. To determine a valid marker, adequate information (e.g., information derived from conducting an adequate metabolism study) is required that relates the proportionate amount of each of the residues to the total residue and their change in quantitative relationships with time. The marker must be a known proportion of the total residue when the total residue depletes to the "safe concentration" in the target tissue. It also must be structurally identified so that an adequate and specific method of analysis can be developed for its determination.

This information must be provided for milk. It would also be required for edible carcass tissues if systemic absorption occurs. Because the resulting residues in milk may be different from the residues in edible carcass tissue, it is possible that a marker compound for milk may be different from a marker compound in edible carcass tissue. Norwich has not provided the necessary information from which a valid marker can be selected to monitor total residues in either milk or edible carcass tissues. The regulatory method proposed by Norwich to control residues in milk uses furaltadone as the marker. Norwich has not substantiated that the measurement of furaltadone is adequate to control total residues of potential concern in milk or alternatively to show that all other residues are safe and do not need to be controlled.

3. Determination of the lower limit of measurement required for the analytical method: Establishment of the lower limit of measurement required for the assay begins with the availability of adequate toxicological testing to determine the cumulative effects of exposure. As described in section I and in steps 2 and 3 of this section, the sponsors have not provided adequate testing. Therefore, an estimate of the acceptable level of exposure to total residues in food cannot be determined.

If it were shown by adequate testing that furaltadone (NF-260) and furazolidone (NF-180) were of comparable carcinogenic potencies, and if it were shown that the estimate of the carcinogenic potency for the total residue could be based on the testing results of the parent drug without the need for further testing of metabolites, then the estimate of the maximum human exposure in the daily diet that could be deemed insignificant for purposes of establishing the minimum assay requirements would be about 0.5 part per billion (ppb). The derivation of this estimate is described in detail in the May 13, 1976 FEDERAL REGISTER notice for furazolidone (NF-180).

Milk may comprise essentially the entire diet of infants. For this reason, no additional adjustments to this amount of daily consumption would be permitted and, under the same assumptions, 0.5 ppb would be the maximum allowed con-

centration of total residues from furaltadone treatment in milk.

No information has been provided on the composition of residues in milk or any measurement of total residues in milk. Therefore, the essential information required to determine a marker for measuring total residues in milk, as described above, is not available. Without this information, even if it were assumed that 0.5 ppb of total residues is "safe," it is not possible to determine the concentration of the marker at the time when total residues in milk would be below 0.5 ppb

Norwich has proposed that the measurement of furaltadone is adequate to assure control of residues, but it has not provided any information to show if furaltadone may be, for example, 80 percent of the total residue or 10 percent of the total residue. Therefore, it is not possible to determine at what concentration the marker must be measured to assure that total residues are at a "safe concentration."

After making the adjustment for the percent of total residue that the marker represents, one further adjustment is necessary to determine the lower limit of measurement required for the assay. This last adjustment takes into account the degree to which the method of analysis recovers the residue from the sample. Minimum standards for recovery for the acceptance of the method take into account that 100 percent recovery is rarely achieved and that a combined evaluation of the precision and accuracy for measurement of the marker must be considered.

Even if very liberal assumptions were made to accept that furaltadone (NF–260) would not be a more potent carcinogen than furazolidone (NF–180) and would be the most potent of the residue compounds that required testing, and if it was determined that furaltadone was a valid marker and was a major constituent of the total residue, and that an analytical method would achieve good recovery of furaltadone, even then it is likely that a method that can measure 0.2 ppb of furaltadone would be required as a minimum to assure safe use.

Under similar assumptions, even if they could be made, methods of analysis in tissue would be required to measure residues at 1 ppb or less in lieu of data demonstrating that no systemic absorption occurred from the udder.

Norwich is aware of the principles just described for the selection of a target tissue, marker compound, and the adjustments required to establish conditions for the assay; this is evidenced by Norwich's citation of a paper prepared by two FDA scientists in a submission for furazolidone (NF–180) to FDA on November 11, 1975. The cited paper by M. K. Perez and N. E. Weber, entitled "Requirements for Metabolic Studies—Impact on Food Safety Regulation" was presented at the Gordon Research Conference on Drug Metabolism, June 23–27, 1975.

The Director concludes that he cannot make an assessment of the toxicity, including the carcinogenic potency, of furaltadone without adequate testing data; he cannot conclude that furaltadone serves as a valid marker for control of total residues of toxicological interest; and therefore he cannot without adequate supporting data determine the conditions necessary for the analytical method used to control residues. The requirements of step 4 have not been fulfilled. He concludes that the new evidence on furaltadone (NF–260) and furazolidone (NF–180) leads to a determination that the methods of analysis for residues for furaltadone (NF–260) are not shown to be adequate.

E. *Practicable Methods of Analysis are Not Available to Assure that Total Residues in the Daily Diet of Humans Do Not Exceed a Level Deemed Safe (Step 5).* Section 514.1(b)(7) (21 CFR 514.1(b)(7)) requires the sponsor of a new animal drug application to provide a practicable method of analysis to be used for the control of residues of its product. A "practicable" method is defined by FDA as one that reliably measures the compound throughout all residue levels of interest, and also is sufficiently rapid and reasonable in terms of the expertise and equipment required to be applicable for use in the surveillance and control of residues. "Reliability" is a combination of accuracy, precision, and specificity. Accuracy and precision are quantitative terms. "Accuracy" is the measure of deviation from the true value, and "precision" refers to the variability of measurement in replications, either by one analyst or within a laboratory or between laboratories. A method may provide reliable measurement if the measurements are not too variable, even though it measures less than or more than 100 percent of the true value. Therefore, accuracy and precision must be considered together.

"Specificity" is a qualitative term. The measurements obtained by the method must reflect only the compound of interest. Because the method will be used on samples from animals for which exposure history is unknown, the techniques used in the method must be adequate to assure not only the integrity of the measurements, but also the identity of the residue to the exclusion of other interfering substances. Therefore, when the quantitative measurements are made by techniques that are not inherently specific, a confirmatory test is required as part of the official method to assure the identity of the residue.

The submitted methodology for furaltadone is based on chromatographic separation and fluorometric determination, which are both nonspecific techniques.

Before receiving reports that furaltadone induced tumors in female rats, FDA had accepted a colorimetric method of analysis for furaltadone, which could detect furaltadone in milk as low as 10 ppb. This method was accepted based on a desk review of recovery data provided by Norwich on the analysis of milk samples to which known amounts of the drug were added (spiked samples). At that time FDA did not require validation trials of methods before acceptance and also did not require evidence that the method satisfactorily recovered the residues when they were naturally incurred through drug treatment (dosed samples).

After FDA received reports of the tumorigenic effects of furaltadone, the drug sponsors were advised in 1969 that their method for determining residues in milk was no longer adequate and that a method capable of determining 0.2 ppb of the drug in milk was required. At a meeting with FDA on January 4, 1971, Norwich representatives stated that they had developed an analytical method for furaltadone (NF–260) in milk capable of detecting 0.2 ppb and had analytical methods for furazolidone (NF–180) in tissue capable of detecting to 2 ppb, which they felt could be adapted to provide acceptable methods for the other nitrofurans.

Both methods were submitted to FDA for evaluation on January 31, 1971. In 1971, the current practice of subjecting potential regulatory methods to validation trials in U.S. Department of Agriculture (USDA) and/or FDA laboratories was in effect.

A discussion of the results on the evaluation of the furazolidone (NF–180) method for tissue analysis is presented in the May 13, 1976 FEDERAL REGISTER notice for furazolidone. In that notice the Director concluded that the methods for edible tissue were not shown to be adequate. No data have been supplied to show that methods of analysis for furazolidone (NF–180) in tissue are adaptable for furaltadone (NF–260). No methods for furaltadone (NF–260) in edible tissues have been provided, except for a method with a claimed lower limit of measurement of 0.1 part per million (ppm), which FDA does not consider to be adequately sensitive

The method presented by the sponsors for milk in 1971 was a thin layer chromatography (TLC) ultraviolet-based method which the sponsors claimed was capable of detecting 0.2 ppb of furaltadone (NF–260). This method was found deficient in a desk review based on the supporting data supplied by Norwich. Subsequently, a revised procedure using TLC–fluorometry for the determination of furaltadone in milk was submitted to FDA and was accepted for laboratory validation trials on May 26, 1972.

The method presented was not quantitative. Norwich and FDA agreed that any positive reading where a peak was observed at the same R_f value as the furaltadone standard would be taken as indicative of a violative residue. (R_f is defined as the ratio of the distance traveled by the solute to the distance traveled by the mobile phase.)

Two FDA laboratories participated in evaluation of the revised TLC-fluorometric analytical method for furaltadone residues in milk. A total of five replicate analyses were performed in the two laboratories on samples of milk from unmedicated cows before and after fortification by the analyst with 0.2 ppb

of furaltadone. Before fortification, four control milk samples produced a zero response (one control sample was lost). Recoveries from milk fortified at the 0.2 ppb level were found to be highly variable, ranging from 10 percent to 55 percent. It was concluded, on the basis of these results, that the method was capable of detecting, but not quantitating, the presence of furaltadone in milk at 0.2 ppb.

Norwich was informed of the results of the validation trial in conferences and of the FDA rejection of the assay method for furazolidone (NF–180) in tissue. Norwich was informed that methods for furaltadone (NF–260) in tissue were required and that a confirmatory method was required for both milk and tissue to provide adequate identification of the residue. The inherent lack of specificity of the TLC-fluorometric method of analysis for furaltadone (NF-260) and the increased probability of unpredictable interference from impurities in solvents, reagents, etc., when measuring residues at extremely low levels, combine to make the need for confirmatory tests for furaltadone in milk and tissues especially acute.

In 1974, as part of its annual Drug Experience Report to the Bureau of Veterinary Medicine, Norwich submitted results of a residue depletion study of furaltadone (NF–260) in milk conducted using a modification of the previously submitted analytical method. The modification consisted of the use of a new technique called programmed multiple development (PMD), which should, in theory, improve the results obtained by thin layer chromatography as it is used in the analysis of furaltadone. However, Norwich reported that it had to discontinue use of the PMD technique in this experiment because of the presence of an extraneous blue fluorescing band that overlapped the critical zones and interfered with the measurement of the drug.

The Director concludes that practicable methods of analysis for milk and tissue residues of furaltadone are not available. No adequate methods have been provided for the analysis of tissue, nor have data been submitted to show cause for exemption from the need for control of residues in edible carcass tissues. Appropriate data have not been provided to determine if furaltadone can be used as a marker to control total residues in milk, nor to determine the lower limit of measurement required for a method to assure that total residues do not exceed safe levels. The Director concludes that the method provided by Norwich for the determination of furaltadone in milk at 0.2 ppb is not quantitative and is not specific and, therefore, is not a quantitatively or qualitatively reliable practicable method for regulatory use, even if it were established that measurement of furaltadone at 0.2 ppb was adequate to control total residues. Therefore, step 5 and the requirements of section 512(b)(7) of the act have not been met.

F. *Practical Conditions of Use Cannot be Prescribed on the Label of Products to Assure Safe Use (Step 6).* Section 512(b)(6) of the act requires that conditions of use assure there are no unsafe residues, and section 512(d)(2)(D) of the act adds a further requirement that these conditions be such that they are "reasonably certain to be followed in practice."

Norwich has provided data that demonstrate that furaltadone is present in milk after use of the drug, and the milk must be discarded for a period of time because of the presence of residues. No data have been provided to show if residues occur in tissues or if a withdrawal period following drug use is required for residues in edible tissues to deplete to safe levels.

A withdrawal period is the time following drug treatment when because of residues, a treated animal cannot be slaughtered for food and its milk must be discarded. A withdrawal period must be sufficiently long to assure adequate residue depletion, but it cannot be so long that it is impractical and would not likely be followed in practice. The maximum withdrawal period considered practical for milk is 96 hours or the discard of eight successive milkings.

It is required by FDA that residue depletion studies be conducted for each drug use under simulated field use conditions to develop data for establishing the withdrawal period. An adequate analytical method for residues, preferably the same method that will be approved for regulatory use, must be used to measure the residues in the depletion studies.

For mastitis products, the residue depletion studies in milk should be conducted with normal lactating cows (disease-free) and mastitic cows to determine if the disease state produces a different rate of residue depletion. The drug treatment in the study must be at the maximum dose for the drug use.

A total of 11 studies of the depletion of furaltadone in milk, using a chemical method of analysis for furaltadone, has been presented to FDA.

Three of these studies used methodology with a lower limit of measurement of furaltadone in milk of 1.0 ppm. Several milk samples collected at 32 hours following medication were shown in these studies to contain levels of furaltadone in excess of 1.0 ppm.

Three more studies used chemical methods with a lower limit of measurement of furaltadone in milk of 10 ppb. On the basis of the results from these experiments, FDA required a 36-hour or three-milking discard period for milk from animals medicated with furaltadone as a condition of approval of the NADA's in 1962.

The following five studies were submitted since the approval of the NADA's. In these studies the chemical methods of analysis used are those described in the preceding section, which Norwich claimed were capable of detecting furaltadone as low as 0.2 ppb.

1. NADA 12–738, Vol. 5, Letter from Norwich dated March 15, 1971.

A single lactating cow with mastitis was treated at the label-recommended dose with furaltadone. Milk collected at 24, 36, 48, and 60 hours after medication was reported to contain furaltadone at 11 ppb, 3 ppb, 1 ppb and less than 0.2 ppb at the respective withdrawal periods. The method of analysis used was presumably the TLC ultraviolet-based method that was judged to be inadequate, on the basis of erratic recovery data, in a desk review by FDA. No supporting raw data were presented. No explanation was given of the significantly lower levels of furaltadone observed in 24- and 36-hour milkings from this cow compared to those found in previously reported studies using less sensitive methodology.

2. NADA 12–738, Vol. 5, Project No. 469–02, dated June 22, 1971.

Five healthy lactating cows were treated with Altapen, a product containing procaine penicillin G in addition to furaltadone. The furaltadone dose was identical to that recommended for formulations containing only furaltadone, and the penicillin was shown not to interfere with the analysis of furaltadone. Milk was collected every 12 hours from each cow, and samples were analyzed for furaltadone using the TLC ultraviolet-based method of analysis that was judged to be inadequate, on the basis of erratic recovery data, in a desk review by FDA. Levels of furaltadone in milk were reported by Norwich to fall from a range of 180 ppb to 3,500 ppb in the five 12-hour post-medication samples, to a range of 1.4 ppb to less than 0.2 ppb in the 48-hour samples. Furaltadone in milk was reported by Norwich to be below 0.2 ppb in all five 60-hour milk samples.

3. Project No. 469.02 and 469.11, Special Report, dated October 25, 1971. Hearing Clerk Docket No. FDC–D–283.

Five dry cows were given two injections of Altapen, 5 days apart, into each udder segment. Cows were not milked until they calved—between 1 and 2 months after the second injection. No furaltadone was found in the first (24-hour) post-parturition sample of colostral milk. The method of analysis was the same TLC ultraviolet-based method discussed under study 2 above.

4. Project No. 469.02 and 469.11. Special Report, dated October 29, 1971. Hearing Clerk Docket No. FDC–D–283.

Five lactating cows (disease state unknown) were treated with a preparation containing only furaltadone according to label-recommended directions. Milk samples were collected every 12 hours, beginning 24 hours after the last injection. Three of the five cows produced 48-hour milk samples reported to contain 0.26 to 0.4 ppb of furaltadone. The method of analysis was the TLC ultraviolet-based method discussed under study 2 above.

5. NADA 12–738–34A, Drug Experience Report submitted to FDA November 1975.

This report contains the results of three residue studies of furaltadone in milk following medication of five mastitic cows in each study with a pathogen-

free formulation of the product. (The drug is marketed as a sterile formulation.) Norwich used the PMD modification of the TLC fluorometric method of analysis discussed under paragraph E (step 5) of this section.

In the first study, furaltadone at levels ranging from 0.2 ppb to more than 24 ppb was found in the five 108-hour post-medication milk samples. Norwich blamed probable contamination of the collection equipment for these results.

In the second study, the milk samples at 72- or 84-hour withdrawal from all five treated cows were reported to contain less than 0.2 ppb of furaltadone. However, later milkings from three of the five animals were reported to contain 0.4 to 1.6 ppb of furaltadone. Norwich blamed these unusual results on improper sampling techniques.

In the third study the PMD modification of the method subjected to validation in 1971 was used initially, but it was discontinued because of the presence of an extraneous blue fluorescence band that overlapped the critical zones and interfered with the measurement of furaltadone. The study was completed using the TLC fluorometric method that was previously determined by FDA in method trials to be capable of detecting, but not quantitating, furaltadone at 0.2 ppb. Furaltadone was found in one of five 84-hour post-medication milk samples. Furaltadone was not found in the milk collected at 96, 108, and 120 hours following drug treatment.

The Director concludes that all the depletion studies submitted on milk are inadequate for the establishment of a milk discard time that will assure no unsafe residues. His reasons are as follows:

a. Adequate information is not available to determine if measurement of furaltadone can be used to assure adequate depletion of total residues.

b. Adequate information is not available to determine the level of residues that can be deemed safe for human consumption.

c. Data obtained in the various studies on the amount of furaltadone in milk are not consistent. For this reason, FDA cannot rely on results of any one of these experiments to predict the levels of furaltadone that may be present in milk from furaltadone-treated cows.

d. The methods of analysis that are claimed by Norwich to be capable of detecting 0.2 ppb of furaltadone are not quantitative at this level. Results at the limit of detection are of necessity extremely variable by definition. The agency can agree only that any positive reading would indicate the possible presence of furaltadone in a milk sample, but it cannot agree that a reading equivalent to less than 0.2 ppb (or a reading below the average reading obtained with samples fortified at 0.2 ppb) as used by Norwich denotes the absence of furaltadone.

The Director concludes that withdrawal periods are required for the safe use of furaltadone; that for products containing only furaltadone, as the active drug, the officially approved withdrawal period for milk of 36 hours or three-milkings discard is not shown to be adequate; that the currently labeled withdrawal period for milk voluntarily used by the sponsors, i.e., 48 hours or four-milkings discard, has not been shown to be adequate; and that for products containing furaltadone and penicillin, the withdrawal period for milk of 96 hours or eight-milkings discard has not been shown to be adequate in light of new evidence presented since the uses of furaltadone were approved. The Director further concludes that withdrawal periods may be required for dairy cows culled from the herd to assure adequate residue depletion from edible tissues before slaughter. He concludes that adequate information has not been provided to show what withdrawals are required for milk discard or slaughter to assure safe conditions of use for furaltadone, as required by section 512(b)(8) and (d)(2)(D) of the act. The requirements of step 6 have not been fulfilled.

Therefore, the Director is hereby withdrawing the notice of opportunity for hearing issued August 4, 1971, proposing to withdraw approval of NADA's providing for the use of furaltadone in food-producing animals. Under section 512(e)(1)(B) of the act (21 U.S.C. 360b(e)(1)(B), the Director proposes to withdraw approval of certain existing NADA's providing for the use of furaltadone in food-producing animals on the grounds that such use violates section 512 of the act in that such use has not been shown to be safe.

This notice applies to all outstanding NADA's for the use of furaltadone in food-producing animals. All such applications known to FDA are listed below by NADA number and by each applicant's name and last known address in FDA records. Copies of this notice are being forwarded by registered or certified mail to each of the known applicants. Notice to other applicants or holders of NADA's whose identity is not known to FDA is given by this publication.

12-738, 65-059, Eaton Laboratories, Division of Morton-Norwich Products, Inc., P.O. Box 181, Norwich, NY 13815.

14-283, Hess and Clark, Division of Rhodia, Inc., 7th and Orange St., Ashland, OH 44805.

Any holder of an approved application that elects to avail itself of an opportunity for hearing pursuant to section 512(e)(1) of the act and § 514.200 of Chapter I of Title 21 of the Code of Federal Regulations must file with the Hearing Clerk, Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, MD 20852, a written appearance requesting such hearing by September 16, 1976, giving the reasons why approval of the application should not be withdrawn, and providing a well-organized and full-factual analysis of the scientific and other investigational data such holder is prepared to prove in support of its opposition to the Director's proposal. A request for hearing may not rest upon mere allegations or denials but must set forth specific facts showing that there is one or more genuine and substantial issue(s) of fact requiring a hearing. Responses to this notice and copies of published literature cited in this notice may be seen in the office of the Hearing Clerk, Food and Drug Administration, during regular business hours, Monday through Friday.

If a hearing is requested and is justified by any holder's response to this notice of opportunity for hearing, the issues will be defined, an administrative law judge will be assigned, and a written notice of the time and place at which the hearing will commence will be issued as soon as practicable.

Any hearing on the withdrawal of approval of the foregoing NADA's will be open to the public. If, however, the Director finds that portions of the applications that serve as a basis for such a hearing contain information concerning a method or process that is entitled to protection as a trade secret, the part of the hearing involving such portions will not be public, unless the respondent so specifies.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (sec. 512, 82 Stat. 343-351 (21 U.S.C. 360b)) and under authority delegated to the Commissioner (21 CFR 5.1) and redelegated to the Director of the Bureau of Veterinary Medicine (21 CFR 5.29) (recodification published in the FEDERAL REGISTER of June 16, 1976 (41 FR 24262)).

A discussion of the economic and environmental impact of this action is set forth in the notice of proposed rule making on furaltadone published elsewhere in this issue of the FEDERAL REGISTER.

It is hereby certified that the economic and inflation effects of this proposal have been carefully evaluated in accordance with Executive Order 11821.

Dated: August 6, 1976.

C. D. VAN HOUWELING,
Director, Bureau of Veterinary Medicine.

[FR Doc.76-23621 Filed 8-10-76;11:19 am]

[Docket No. 76N-0232]

HESS & CLARK, ET AL.

Nitrofurazone (NF-73); Opportunity for Hearing on Proposal To Withdraw Approval of Certain New Animal Drug Applications

The Director of the Bureau of Veterinary Medicine of the Food and Drug Administration (FDA) is issuing a notice of opportunity for hearing on a proposal to withdraw approval of approved new animal drug applications (NADA's) Nos. 6-395, 6-475, 6-649, 7-801, 8-129, 8-142, 8-324, 8-410, 8-529, 8-784, 9-013, 9-415, 10-741, and 11-154 for the use of nitrofurazone (NF-7) in food-producing animals in accordance with section 512(e)(1)(B) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b(e)(1)(B)) (hereinafter, "the act") on the grounds that new evidence not available when the applications were approved, evaluated together with the evidence available at the time of approval, shows that nitro-

furazone is not shown to be safe for use under either the approved or currently labeled conditions of use. Holders of approved NADA's have until September 16, 1976 to submit requests for a hearing in accordance with § 514.200 (21 CFR 514.-200).

In FR Doc. 76-23620 appearing elsewhere in this issue of the FEDERAL REGISTER, the Director is issuing a notice of proposed rulemaking to revoke the appropriate provisions of sections affected by the proposed withdrawal.

Nitrofurazone (NF-7) is a member of a class of chemicals called "nitrofurans," which are used at low levels in animal feed as antibacterial and antiprotozoan agents to increase resistance to disease, thereby assisting growth. Nitrofurans also have prescribed therapeutic uses. In addition to nitrofurazone (NF-7), which is the subject of this notice, there are three other nitrofurans that are used in food-producing animals and are or have been subject to agency action: nihydrazone (NF-64) and furaltadone (NF-260), which are subjects of companion notices in this issue of the FEDERAL REGISTER; and furazolidone (NF-180), which was the subject of notices in the FEDERAL REGISTER of May 13, 1976 (41 FR 19906, 19907). A fifth nitrofuran, furamazone (NF-84), is not at this time the subject of contemplated agency action for withdrawal.

Nitrofurazone (NF-7), approved in 1948, was the first nitrofuran approved by FDA for use in food-producing animals. It is currently approved for use in mastitis products for dairy cattle (dry cow treatment only); for the treatment of genital tract infections in large female animals; for prevention and treatment of surface bacterial infections (all animals); as a medication in feed for swine for the treatment of infectious necrotic enteritis caused by *Salmonella choleraesuis;* for chickens and turkeys for the prevention and control of cecal and intestinal coccidiosis; and for chickens for the prevention of pullorum.

The original applications were approved without a requirement that the drug treatment be discontinued for a specified period of time before the slaughter of the animals. However, two of the sponsors, Norwich Pharmacal Co., Division of Morton-Norwich Products, Inc., and Hess & Clark, currently a Division of Rhodia, Inc., voluntarily revised the labels in 1971 to require that swine, turkeys, and chickens be withdrawn from nitrofurazone (NF-7) 5 days before slaughter.

The Food and Drug Administration issued a notice of opportunity for hearing, published in the FEDERAL REGISTER of March 31, 1971 (36 FR 5927), proposing to withdraw approval of NADA's for nitrofurazone (NF-7) on grounds that:

Information available to the Commissioner establishes that the drugs, when administered to laboratory animals, have been shown to produce tumors. The drugs are, therefore, not considered to be safe for use in the absence of appropriately sensitive methods of analysis to establish their absence in food derived from treated animals.

Similar notices were published for nihydrazone (NF-64) in the FEDERAL REGISTER of March 31, 1971 (36 FR 5926) and for a hearing. Norwich assured FDA that done (NF-260) on August 4, 1971 (36 FR 14343). The history leading to the publication of these 1971 notices is discussed in the May 13, 1976 notice for furazolidone (NF-180).

Norwich and Hess & Clark responded to the March 31, 1971 notice concerning nitrofurazone (NF-7) by filing requests for a hearing. Norwich assured FDA that it had adequate methods of analysis to determine residues and adequate data to show that under the currently labeled conditions of use, the drugs were safe. In addition, Norwich set forth a hypothesis that nitrofurans were not primary tumorigens but stimulated spontaneous tumor growth by disrupting the normal hormone balance.

In response to the March 31, 1971 notice, Pharm-House, Inc. submitted a method for the determination of nitrofurazone (NF-7) in milk, which it indicated could detect nitrofurazone at levels down to 2 parts per billion (ppb).

Norwich has continued to submit data pertaining to the use of nitrofurazone (NF-7) in food-producing animals. The Food and Drug Administration has determined that new evidence, evaluated together with the evidence available when the approvals were granted, fails to demonstrate that either the approved or currently labeled uses of nitrofurazone are shown to be safe. The Director is withdrawing the March 31, 1971 notice of opportunity for hearing concerning nitrofurazone (NF-7), and is issuing this new notice, which presents in detail all of the evidence now available to him and the basis on which he proposes to withdraw approval of all uses of nitrofurazone in food-producing animals in accordance with section 512(e)(1)(B) of the act.

Section 512(e)(1)(B) of the act requires that FDA withdraw approval of an NADA when "new evidence * * * evaluated together with the evidence available * * * when the application was approved, shows that such drug is not shown to be safe * * *." Under this provision, the new evidence and the evidence submitted at the time the application was approved must be evaluated to determine whether all of the requirements for safety contained in section 512 of the act continue to be fulfilled.

THE DETERMINATION OF "SAFETY" IN SECTION 512

Section 512 of the act requires that the use(s) of a new drug in food-producing animals must be supported by adequate data supplied by the sponsor in applications that include, among other things, demonstration that the drug use will not result in unsafe residues in human food.

Under section 512(b) of the act, whenever a drug use may result in residues of the drug in food, an application must include not only full reports of investigations to show that the use of the drug is safe, but also a description of practicable methods for monitoring food to assure there are no unsafe residues in human food attributable to the drug use, and demonstration that the conditions of use are adequate to assure there are no unsafe residues.

Under section 512(d)(2) of the act, FDA is required, in the evaluation of the supporting safety data, to consider, among other things:

1. The probable consumption of such drug and of any substance formed in or on food because of the use of such drug (i.e., probable human consumption of residues, including the parent drug and metabolites).

2. The cumulative effect on man or animal of such drug, taking into account any chemically or pharmacologically related substance, i.e, toxicological effects of the compounds comprising the residues.

3. Safety factors that, in the opinion of experts qualified by scientific training and experience to evaluate the safety of such drugs, are appropriate for the use of animal experimentation data (i.e., establishing "safe" levels of residues using appropriate safety factors to extrapolate animal data on cumulative effects to humans).

Under section 512(d)(1)(H) of the act, additional requirements must be met if the drug is a carcinogen.

The determination of safety within the meaning of section 512 of the act for a drug whose use may result in residues in the edible products of the treated animal, whether or not the drug is a carcinogen, requires a multistep process. These steps form an orderly procedure by which information is derived to satisfy each of the three considerations stated above.

Step 1. Assessment of the total residues in edible products and the depletion of those residues with time following cessation of drug treatment in order to determine probable human consumption (section 512(b)(1) and (d)(2)(A) of the act).

Step 2. Assessment of information about the residues to determine the appropriate testing in laboratory animals required to estimate the cumulative effects of human exposure to the residues (section 512(b)(1) and (d)(2)(A) of the act).

Step 3. Acquisition of dose-response data in test animals to determine the cumulative effects of dietary exposure to the residue compounds from which the maximum acceptable concentration of residues in food can be established using appropriate safety factors to assure that human exposure to residues in the total diet does not exceed a level deemed safe (section 512(b)(1) and (d)(2)(B) and (C) of the act). In the case of a carcinogenic drug, a conservative statistical extrapolation of the tumor data is used to estimate a level of residue exposure that could be considered insignificant and poses essentially no risk of cancer and operationally defines "no residue" for purposes of computing the residue assay requirements under section 512(d)(1)(H) of the act.

Step 4. Acquisition of information about the residues to (1) determine the tissue (called the target tissue) from which residues require the longest time to deplete to safe levels and which, therefore, must be monitored by the regulatory assay (step 5), and (2) determine the compound (called the marker) that is to be measured in the regulatory assay so that by its measurement there is assurance that no unsafe residues occur from the use of the drug (section 512(b)(7) of the act).

Step 5. Development of a practicable method of analysis that can measure the marker in the target tissue at a concentration that assures that total residues in the daily diet of humans do not exceed the level established in step 3 as safe (section 512(b)(7) of the act).

Step 6. Demonstration that practical conditions of use can be prescribed on the label of the product to assure there will be no unsafe residues in the food products from treated animals (section 512(b)(8) and (d)(2)(D) of the act).

Each of the steps involves principles that are essential for a determination of food safety and compliance with the requirements of the act to assure there are no unsafe residues in human food from the animal drug use. Each step builds upon the preceding step. Therefore, failure to accomplish any one of the six steps in whole or in part for any drug will ordinarily preclude a sponsor's fulfilling its obligation under the act to determine probable consumption of residues and cumulative effects of exposure, and to establish safe conditions of use with appropriate regulatory methods of analysis to control residues when deemed necessary.

New evidence concerning nitrofurazone (NF–7) not available when the applications were approved includes, among other things, a demonstration that nitrofurazone induces mammary tumors in female rats. The Director has evaluated all the data now available, including the new data received since 1948 when the uses for nitrofurazone (NF–7) were first approved, and the data available to FDA at the time of approval, and has concluded that the approval of NADA's for nitrofurazone (NF–7) must be withdrawn under section 512(e)(1)(B) of the act because:

1. Adequate investigations have not been provided to show that the uses of nitrofurazone are safe, as required by section 512(b)(1) of the act. Adequate information has not been provided on the probable consumption of residues, and the cumulative effects of exposure have not been determined by appropriate tests, as required by section 512(d)(2)(A) and (B) of the act. Therefore, "safe" levels of residue cannot be determined using appropriate safety factors, as required by section 512(d)(2)(C) of the act.

2. Adequate, reliable, and practicable methods of analysis are not available for monitoring food to assure there are no unsafe residues in edible tissues and milk that are attributable to the use of nitrofurazone, as required by section 512(b)(7) of the act.

3. Practical conditions of use cannot be specified in the labeling to assure there are no unsafe residues attributable to the use of nitrofurazone, as required by section 512(b)(8) and (d)(2)(D) of the act.

The data on which these conclusions are based are presented in the succeeding sections of this notice. The Director first describes all the relevant new evidence bearing on the determination of safety for the uses of nitrofurazone. Then he evaluates all the evidence before him using the six-step procedure necessary to satisfy a human safety determination for the use of nitrofurazone (NF–7) and to meet the considerations required in section 512 of the act.

I. New Evidence

In In October 1966 and in January 1967, Norwich submitted reports of the effect in rats of feeding diets containing nitrofurans for 45 weeks. These studies included tests on nitrofurazone (NF–7) and furazolidone (NF–180). In these studies it was shown that both nitrofurazone (NF–7) and furazolidone (NF–180) induced mammary tumors of similar type in female rats.

Subsequently, more extensive, longer term testing of furazolidone (NF–180) was conducted, but further testing of the carcinogenic potential of nitrofurazone (NF–7) has not been undertaken. The more extensive testing of furazolidone (NF–180), as described in the May 13, 1976 Federal Register, shows that the drug induces cancer in both rats and mice and that the tumors and cancers were not limited to the mammary gland or the endocrine tissues of female rats.

The Director is including in this notice information available on the toxicological properties of furazolidone (NF–180) as well as introfurazone (NF–7) because of the structural similarities of the drugs and their similar biological properties, which have been observed in the limited comparative data available. The new evidence showing that furazolidone (NF–180) is a carcinogen has a significant bearing on the Director's conclusions that the uses of nitrofurazone (NF–7) are not shown to be safe.

Although it is appropriate to consider data on furazolidone (NF–180) to judge the adequacy of existing data to support the safe use of nitrofurazone (NF–7) in food-producing animals, these data are not a substitute for data on nitrofurazone (NF–7) that are required by the statute. Basic data are required for each drug, recognizing that slight differences in chemical structure can result in significant differences in metabolism, residues, and toxicity. Although comparative data or the biochemical and toxicological properties of related drugs may be useful and appropriate in making decisions about the degree of testing required for each drug to show there is no probable hazard, these data cannot be used as a substitute for the basic information necessary on each drug to determine the probable exposure to residues and cumulative effects of exposure.

A. *Carcinogenic Potential of Nitrofurazone.* Norwich submitted data in October 1966 and January 1967 on the cumulative effects in rats of feeding diets containing nitrofurazone (NF–7) for 45 weeks ("Effects of Long Term Feeding of Various Nitrofurans," October 13, 1966 and January 4, 1967, Project No. 440.07, submitted to FDA as part of a June 2, 1967 Drug Experience Report). These studies include testing in female Sprague-Dawley (Holtzman strain) rats and testing in both male and female Carworth Farms (CFE strain) rats.

1. The Sprague-Dawley Female Rat Study: Seventy female Sprague-Dawley rats (Holtzman strain) were equally divided into two groups that were fed nitrofurazone at about 0.1 percent (55 milligrams per kilogram (mg/kg) body weight per day) and about 0.05 percent (28 mg/kg body weight per day) respectively in their diet for 45 weeks, followed by maintenance on an unmedicated diet for an additional 8 weeks before sacrifice. A comparable control group of female rats was maintained on a nitrofurazone-free diet for 53 weeks. At termination of the study the animals were autopsied, and tissues were examined for histopathological lesions.

The most prominent drug-related effect was the induction of tumors in the mammary glands of the female rats. Although rats develop mammary tumors spontaneously as they age, the incidence of spontaneous mammary tumors in rats just over 1 year old, depending upon the strain of rat used, can be quite low. The nitrofurazone-treated female rats had an increased number of mammary tumors, particularly the number of fibroadenomas, as compared with the control rats. The development of tumors included the finding of some mammary adenocarcinoma (cancer).

Norwich reported that no mammary tumors of any type were observed in the 33 control rats examined. It reported from hispopathological examination that a total of 19 mammary tumors were observed in 13 of the 30 rats examined that were fed nitrofurazone at 0.1 percent in the diet. In the 0.05 percent dose group a total of 18 mammary tumors were found by Norwich in 12 of the 33 rats examined for histopathology. Norwich did not report any of adenocarcinoma in either the 0.1 percent or 0.05 percent dose groups.

An Ad Hoc Committee was established to review the pathology data on all the nitrofurans tested in the Sprague-Dawley rat study and the Carworth Farms rat study. One member of the Ad Hoc Committee was the director of the Eppley Institute for Research in Cancer, University of Nebraska. He and his associate pathologists reviewed the pathology slides of the mammary tissues from the nitrofurazone-treated rats provided by Norwich and submitted a detailed report to FDA. They did not review all the mammary gland slides from the control rats.

Each of the reviewed slides had a rat identification number, and there were several slides for each rat. However, there was no information on the number of tumors in each rat. Therefore, it was not possible, in many cases, for the pathologists to determine which slides represented different tumors in the same rat and which were different sections of a single tumor.

The Eppley patholigists concluded from their review of the 0.1 percent dose group rats that 14 of the 30 nitrofurazone-treated female rats examined had mammary tumors that were predominantly fibroadenomas and that 4 of these rats had mammary adenocarcinoma.

From their examination of the 0.05 percent nitrofurazone dose group, they found mammary tumors in 12 out of the 33 rats examined. This included the finding of mammary adenocarcinoma in one rat. The remainder of the tumors in this dose group were fibroadenomas.

Because the Norwich report did not indicate which rats had tumors, it is not possible from the data available to determine if the tumors reported by the Eppley pathologists were from the same rats in which Norwich had reported tumors. The Eppley pathologists reported mammary adenocarcinoma in the nitrofurazone-treated rats, whereas the Norwich report shows no nitrofurazone-treated rats had mammary adenocarcinoma. However, because the Eppley pathologists did not review all the mammary gland slides from the control rats, a comparative incidence of mammary cancer cannot be determined from the reports.

A pathologist from the Bureau of Foods of FDA, who was not a member of the Ad Hoc Committee, later reviewed the pathology slides of the mammary tumors from the nitrofurazone-treated rats in which the Eppley pathologists had reported mammary tumors. From his examination of the histopathological preparations provided, he reported for the 0.1 percent dose group, 15 slides of mammary fibroadenoma from 10 rats and 4 slides of mammary adenocarcinoma from 3 rats. The mammary adenocarcinomas were diagnosed in the same three rats in which the Eppley pathologists had diagnosed mammary adenocarcinoma. The Bureau of Foods pathologist did not have any slides from the fourth rat in which the Eppley pathologist had found adenocarcinoma. In the 0.05 percent dose group, the Bureau of Foods pathologist reported histopathological preparations of mammary tumors from 12 rats. These included 5 slides of adenoma, 13 slides of fibroadenoma, and 1 slide of adenocarcinoma. The mammary adenocarcinoma was in the same rat in which the Eppley pathologists had diagnosed mammary adenocarcinoma.

The Bureau of Foods pathologist also did not have information indicating which slides were multiple sections of single tumors or which represented different tumors. He did have information on the rat identification number for each set of slides. Because the Bureau of Foods pathologist did not review all the slides in the study, tumor incidence and the comparison of incidence with the control rats cannot be determined from his report. However, his report supports the Eppley pathologists' findings that mammary tumors were found in at least 13 of the rats in the 0.1 percent nitrofurazone-treated group and in at least 12 of the rats in the 0.05 percent nitrofurazone-treated group. It also confirms that mammary adenocarcinoma was found in at least three rats in the 0.1 percent nitrofurazone-treated group and in one rat in the 0.05 percent nitrofurazone-treated group.

2. The Carworth Farms Rat Study: Nitrofurazone (NF–7) was fed to 20 male and 20 female Carworth Farms (CFE strain) rats at about 0.1 percent in the diet (50 mg/kg body weight per day) for 45 weeks, followed by maintenance on an unmedicated diet for an additional 7 weeks before sacrifice. A comparable control group of rats was maintained on a nitrofurazone-free diet for 52 weeks. At termination of the study the animals were autopsied, and the tissues were examined for the histopathological lesions.

As in the previous Sprague-Dawley rat study, the most prominent drug-related effect seen was the induction of tumors in the mammary glands of the female rats. This included an increase in the number of mammary fibroadenomas and the finding of some adenocarcinomas.

Norwich reported that no tumors were observed in the 15 female control and 18 male control rats examined. Norwich reported from histopathological examination that 11 out of 19 nitrofurazone-treated female rats examined had a total of 28 mammary tumors. No mammary tumors were reported in the male rats treated with nitrofurazone. Norwich reported 3 mammary adenocarcinomas among the 28 mammary tumors found in the nitrofurazone-treated female rats but did not specify whether these adenocarcinomas were seen in 3 different rats or whether some of these were multiple tumors in the same rat.

The Eppley pathologists reviewed the slides of the mammary tissues from the 19 nitrofurazone-treated rats provided by Norwich. They also concluded that 11 of the nitrofurazone-treated female rats had mammary tumors. However, from the slides reviewed they found only one rat with mammary adenocarcinomas. It is not known if the Eppley pathologists had all the slides that Norwich had diagnosed as mammary adenocarcinoma.

Slides of the mammary tumors reported by the Eppley pathologists were later reviewed by the Bureau of Foods pathologist. He reported mammary tumors in each of the 11 female rats. From his examination of the histopathological preparations provided, he reported 9 slides of mammary adenoma, 1 slide of mammary fibroma, 19 slides of mammary fibroadenoma, and 1 slide of mammary adenocarcinoma. The adenocarcinoma was in the same rat in which the Eppley pathologist had reported a mammary adenocarcinoma.

Because Norwich did not report the number of rats in which mammary adenocarcinoma was found, and because the Eppley and Bureau of Foods reviewing pathologists did not receive mammary tissue slides from control rats, and because the Bureau of Foods pathologist did not review slides from all of the treated rats, and because there is some question about whether the Eppley pathologists received all the mammary tissue slides of the treated rats, the incidence of mammary cancer cannot be determined from the available reports. However, the Eppley and Bureau of Foods reviewing pathologists did concur in the finding of mammary tumors in 11 rats and mammary adenocarcinoma in at least 1 nitrofurazone-treated female rat from the study.

Food and Drug Administration scientists recognize that there are uncertainties and limitations in the examination of tumors to determine if they have malignant potential. For example, in the early stages of a mammary tumor growth, the mass may be chiefly adenomatous. As the growth continues, the supporting white fibrous connective may also begin to proliferate, resulting in a fibroadenoma. Later the adenomatous part of the neoplastic mass may entirely disappear, leaving a fibroma. Finally, a part of the adenoma or fibroadenoma may develop malignant tendencies and that portion may then become an adenocarcinoma. All four tumor types, i.e., adenoma, fibroadenoma, fibroma, and adenocarcinoma may be present in the same tumor mass. A small focus of adenocarcinoma in the early stage of a malignant tumor may be overlooked in the tumor growth unless adequate multiple sections of the tumor are made.

Also, unless great care is exercised in the evaluation of the spread of an adenocarcinoma of the mammary gland, the metastasis along the lymphatic routes passing through, under, or near other mammary glands may be considered to be separate primary adenocaricinomas rather than a single primary growth with multiple metastases.

The reports from Norwich indicate that nitrofurazone (NF–7) caused an induction of mammary tumors in female rats just over 1 year old and that there is an indication of an increase in the number of mammary adenocarcinomas compared with control rats.

Erturk et al. (*Cancer Research*, 30:1409–1412, 1970) transplanted mammary fibroadenomas from female Holtzman rats orally treated with nitrofurazone into male and female newborn Holtzman rats. They reported increased anaplasia pleomorphism and mitotic activity in the rats into which the tumors had been transplanted. One of the tumors developed into an adenocarcinoma. The transplantability of rat mammary fibroadenomas and their conversion to malignant tumors has been considered by some eminent pathologists as one criterion of cancer (H. L. Stewart et al., "Atlas of Tumor Pathology," Sec. XII,

Fascicle 40, pp. 249–297, Washington, DC, Armed Forces Institute of Pathology, 1959).

Food and Drug Administration scientists conclude that the above studies show that nitrofurazone is tumorigenic in rats and that the data raise questions about the carcinogenic potential and potency of nitrofurazone. They conclude that none of the studies is adequate to assess the cumulative effects of exposure to nitrofurazone and its carcinogenic potential and potency because the studies were not of sufficient duration, and the number of animals used was not sufficient. In the Sprague-Dawley rat study only female animals were used; so it is not possible to determine whether nitrofurazone might have produced effects in the male rats. In the Carworth Farms rat study, the number of males was too small to determine with any confidence that nitrofurazone did not have an adverse effect. In both studies, drug treatment was for only 45 weeks. In spite of the weaknesses in the reports available, it is evident that a 45-week treatment was sufficient to induce tumors in female rats, and that some of these tumors were mammary cancers. The dose level, frequency, and duration of exposure to a compound are related not only to whether or not cancer is induced, but also to the latency period before the expression of cancer. Therefore, the 45-week treatment with nitrofurazone is not considered a sufficient period of exposure to determine adequately the cancer potential or potency.

The Director concludes that the available data provide sufficient cause for concern that nitrofurazone may be a carcinogen and that adequate lifetime testing in appropriate animal species is required to evaluate its carcinogenic potential and potency. The Director concludes that because nitrofurazone is a tumorigen, dose-response data in lifetime studies, using several dose levels, are necessary to determine the information required to assess the cumulative effects of exposure and to establish conditions of safe use. Although the demonstration of cancer induction may be made on the basis of testing at only one dose level, and it may be possible from the results of this testing to conclude that a drug requires regulation under the provisions of section 512(d)(1)(H) of the act, dose-response data are necessary to provide information from which FDA can establish the operational definition of "no residue" for purposes of determining the required conditions for the regulatory assay for residues of a carcinogenic drug. Regardless of whether adequate testing were to show that nitrofurazone induced cancer or induced only benign tumors, dose-response information would be required to establish the condition of safe use.

The Norwich data from these studies were available at the time FDA issued the March 31, 1971 FEDERAL REGISTER notice of opportunity for hearing on the proposed withdrawal of approvals for nitrofurazone (NF–7). In response to the March 31, 1971 notice, Norwich stated that all four nitrofurans (NF–7, NF–64, NF–180, and NF–260) cause an early development of mammary tumors that were of the type normally found in aging rats, and it postulated that the nitrofurans caused these tumors only at high levels of exposure in the female rats because of disruption in the hormonal system. Norwich presented data purporting to show that the hormonal disruption resulting in the tumors involved a process not applicable to humans, and that the proposed methods of analysis for control of residues were more than adequate to assure that levels of residues were below any level that might adversely affect humans.

The Norwich hormonal hypothesis is described in detail in the May 13, 1976 FEDERAL REGISTER notice on a furazolidone (NF–180). The supporting information provided by Norwich for the hypothesis was primarily limited to data on furazolidone (NF–180) and some very limited comparative data on nitrofurazone (NF–7).

B. *Comparative Data on Nitrofurazone and Furazolidone.* Norwich has provided data on furazolidone (NF–180) from the Sprague-Dawley female rat study and the Carworth Farms rat study. It has also supplied data on testing on furazolidone (NF–180) in three chronic rat studies and one mouse study; the tests are described in the May 13, 1976 FEDERAL REGISTER notice. The data have a bearing on the Director's proposed action in this notice for nitrofurazone (NF–7) and his decision to reject the Norwich hormonal hypothesis for nitrofurazone (NF–7) and furazolidone (NF–180). They also have a bearing on his decision to reject the sponsors' claims that adequate studies have been provided for nitrofurazone (NF–7) to support a determination that the drug uses are shown to be safe.

In the Sprague-Dawley female rat study and Carworth Farms rat study, furazolidone (NF–180) was also tested in the protocols using a dietary level of about 0.1 percent.

In the first study, Norwich reported that 14 of the furazolidone-treated Sprague-Dawley female rats out of 32 rats examined had mammary tumors. Norwich reported a total of 37 mammary tumors, including 5 mammary adenocarcinomas, but did not designate in how many rats or in which rats the adenocarcinomas were found. The Eppley pathologists reviewed the mammary tissue slides from the 32 furazolidone (NF–180)-treated female rats and reported mammary tumors in 14 of the rats. The Bureau of Foods pathologist, who later examined only the slides from the 14 rats reported to have mammary tumors by the Eppley pathologists, confirmed the presence of mammary tumors in the 14 rats. Both the Eppley and Bureau of Foods pathologists reported only one mammary adenocarcinoma and one mammary squamous cell carcinoma, each in a different rat, from the slides they had examined of the furazolidone (NF–180)-treated rats. From these results it appears that both furazolidone (NF–180) and nitrofurazone (NF–7) at 0.1 percent in the diet had about the same effect on the incidence of rats that developed mammary tumors. However, Norwich reported that furazolidone (NF–180) caused a greater increase in the number of multiple mammary tumors that developed. From the reviewing pathologists' reports it appears that there were slightly more female rats treated with nitrofurazone (NF–7) that developed mammary adenocarcinomas that were found in the rats treated with furazolidone (NF–180). The results reported by Norwich contradict this finding on malignancies.

In the Carworth Farms rat study, 15 of the furazolidone (NF–180)-treated female rats out of 17 examined were reported by Norwich as having mammary tumors. In comparison, Norwich had reported that 11 of the nitrofurazone (NF–7)-treated female rats out of 19 examined had mammary tumors. In the furazolidone (NF–180) study the total number of tumors reported by Norwich in the furazolidone (NF–180)-treated female rats was greater than twice the number found for the nitrofurazone (NF–7)-treated female rats. Norwich reported finding two mammary adenocarcinomas in the furazolidone (NF–180)-treated female rats, but it did not specify whether these were in one or two rats. The Eppley pathologists also reported finding two mammary adenocarcinomas in two rats, and these same findings were made by the Bureau of Foods pathologist who reviewed only the slides from rats in which tumors were reported by the Eppley pathologists. In addition, the Bureau of Foods pathologist reported mammary adenocarcinoma of low-grade malignancy in two additional rats. Tumors from these two additional rats had been diagnosed as fibroadenoma by the Eppley pathologists. The numer of mammary malignancies reported for rats treated with nitrofurazone (NF–7) and furazolidone (NF–180) are comparatively small in both cases, and no meaningful conclusions can be drawn about the relative carcinogenic potentials of the two drugs. It is evident from the reviewing pathologists' reports that the predominant number of tumors found in the furazolidone (NF–180)-treated female rats were fibroadenomas, as was also seen in the nitrofurazone (NF–7)-treated rats.

Based on these comparative data, the Director concludes that furazolidone (NF–180) and nitrofurazone (NF–7) qualitatively exhibit similar tumorigenic effects in female rats. Because the studies are not adequate to assess the cumulative effects of exposure and carcinogenic potential and potency, the data are not sufficient for a precise comparison of the tumorigenic potencies. The Director, therefore, concludes that although the limited data give some indication that nitrofurazone (NF–7) may be a less potent tumorigen than furazolidone (NF–180), adequate testing of nitrofurazone (NF–7) in lifetime studies in appropriate animal species with the development of dose-response data from the administration of nitrofurazone at

several dose levels would be required to determine adequately the carcinogenic potential of nitrofurazone (NF–7).

More definitive studies of the carcinogenic potential of furazolidone (NF–180) in male and female rats and mice provided by Norwich are described in the May 13, 1976 FEDERAL REGISTER notice. A summary of the findings from the three chronic rat studies and the mouse study include the following:

1. Furazolidone induced malignancies in both rats and mice.

2. Furazolidone also induced nonmalignant tumors.

3. In most cases, the induced malignant and nonmalignant tumors were found at the same sites.

4. There is some evidence that the induction of benign tumors is related to the induction of, or progression to, malignancies as a function of dose.

5. There are marked sex differences in the tumor responses in the rats. There are also some commonalities in the tumor sites of the male and female rats.

6. Mammary tumors were the predominant effect in the female rats.

7. Nonmammary tumors were induced in the Sprague-Dawley male rats, with no one type singularly significant. In the Fisher rats, however, several specific nonmammary tumors were induced in both male and female rats.

8. The major target site for tumors in the mouse was the lung. There was no evidence of a significant difference between male and female mice in tumor response, and there were no tumorigenic effects in the endocrine tissues.

9. The induction of tumors involved not only increased incidence of animals with tumors but also an increased number of tumors per animal.

The Director has rejected the data supplied by Norwich to support the hormonal hypothesis for furazolidone (NF–180). A discussion of these data and his basis for rejection are also presented in the May 13, 1976 FEDERAL REGISTER notice. The Director also rejects the Norwich reply to the 1971 FEDERAL REGISTER notice on nitrofurazone (NF–7) attempting to explain the tumorigenic effects of nitrofurazone primarily on the basis of the furazolidone (NF–180) data relative to the hormonal hypothesis. The Director finds that the new evidence provided by the more extensive chronic testing data for furazolidone (NF–180), which shows that tumors were induced in both male and female rats and mice and were not limited to tumors of the mammary gland or the endocrine tissues, is inconsistent with the hormonal hypothesis.

Therefore, the Director concludes that the comparative data available on furazolidone (NF–180) and nitrofurazone (NF–7) in the Sprague-Dawley female rat study and the Carworth Farms rat study, together with the data on the four chronic studies of furazolidone (NF–180) in rats and mice, indicate that adequate testing of nitrofurazone (NF–7) has not been provided to assess the cumulative effects of exposure necessary for a determination of safety.

II. USES OF NITROFURAZONE ARE NOT SHOWN TO BE SAFE

To assess the safety of residues using criteria described in section 512(b) of the act and elaborated on in section 512 (d)(2) of the act requires a multistep process that must take into account consideration of the metabolism of the drug, determination of the resulting residues, including parent drug and the metabolites, depletion of those residues with time after drug treatment, and the cumulative effect of human ingestion of the residues. The six basic steps involved in this evaluation process have been described previously in this notice.

A. *The Amount of Total Residue in the Edible Products and the Depletion of Residues with Time Has Not Been Determined (Step 1).* When a drug is administered to an animal, the drug may be altered by the animal into a variety of compounds called metabolites. The process of alteration may be such that little residue of the parent drug remains in the animal tissue, but the amount of metabolites may be substantial. Therefore, the total residue with which FDA is concerned includes both the parent drug and the metabolites.

The nature and amount of each of the residues depend in part upon the metabolism in a particular species. Because there are species differences in metabolism, it is necessary to determine metabolism and residues in each of the species for which the drug is used. Therefore, § 514.1(b)(7)(i) (21 CFR 514.1(b)(7)(i)) includes metabolism and residue depletion studies among those kinds of studies that are used to determine residues.

Exposure to nitrofurazone in the diet induced mammary tumors in female rats. It is not known whether the parent drug was the active agent or whether the responses were due to one or more active metabolites produced as a result of ingestion of the parent drug by the rats. Such lack of knowledge is not unique to nitrofurazone; it is typical of many tumorigenic and carcinogenic agents that the mechanism of the induction of tumors or the identification of the active agent is not known. Therefore, when considering the safety of food from nitrofurazone-treated animals, all drug-related residues to which humans may be exposed must be assumed to be potentially carcinogenic unless convincing evidence is presented to the contrary. Such evidence consists of not only the identification of the residues that are claimed to be safe, but also adequate toxicological studies showing that the levels of each of the residues claimed to be safe are in fact safe. All residues not identified or not shown to be safe must be considered potentially carcinogenic and must be required to be below a level of measurement that can be considered insignificant and to pose essentially no risk of cancer in humans.

The use of chemical assays to determine initially the total residues would require knowledge about what residues occur (parent drug and metabolites) and would require the availability of adequate methods of analysis to measure each one of the residues at the level of interest in the edible tissues. Therefore, this chemical approach is usually not feasible. Instead, a radiolabeled parent drug is used to provide a practical and adequate approach to the initial measurement of total residues in the edible tissues. In this approach, the drug is synthesized with a radioisotope so that when the drug is administered to the test animal according to label directions, all resulting residues of potential interest can be measured in the edible products. This is accomplished by counting the radioactivity in the tissues and converting these measurements to an estimation of the amount of residue expressed as parent drug equivalents.

No data have been provided by the sponsors to measure total residues in edible tissues and in milk from nitrofurazone-treated animals. Similarly no data have been provided to characterize the residues occurring in the edible products. It has been shown that nitrofurazone is found as a residue in edible tissues after drug treatment, as will be described below in step 6 (paragraph F. of this section), but it has not been shown what proportion of the total residue is the parent drug.

Although no data of any type have been supplied on the metabolism of nitrofurazone in any of the animals of intended use, some limited information has been provided on nitrofurazone in published studies of the absorption, distribution, and metabolism of nitrofurazone, primarily in rats:

1. Paul, M. F. et al., "Studies on Distribution and Excretion of Certain Nitrofurans," Antibiotics and Chemotherapy, 10:287–302, 1960. The authors reported that only 4.6 percent of an administered dose of nitrofurazone was recovered as unchanged nitrofurazone in rat urine, and 0.5 percent in feces. From the report it is evident that nitrofurazone is absorbed and rapidly metabolized before elimination.

2. Paul, H. E. et al., "Metabolic Degradation of Nitrofurans," Journal of Medicinal and Pharmaceutical Chemistry, 2:563, 1960. The report described in vitro studies where nitrofurazone was added to rat tissue homogenates. After a period of incubation, extracts were analyzed by ultraviolet (uv) spectroscopy. The resulting UV spectra were compared with (1) spectra from nitrofurazone that had been subjected to chemical reduction of the nitro-group to an amine, and (2) nitrofurazone that had been subjected to action by certain reductive enzyme systems. The authors report that the three uv spectra were similar, indicating that nitrofurazone metabolism in the rat appears to include a reductive pathway.

Data from these two studies reinforce the Director's conclusion that consideration must be given to metabolism in the animal of intended drug use to assess the residues and their significance as contaminants in food.

Data on the metabolism of another nitrofuran, nihydrazone (NF–64), in chickens have been submitted by Norwich. However, these data cannot be accepted as a substitute for metabolism data on nitrofurazone in chickens or the other animals for which the drug is approved because the metabolism of each compound may differ in each of the animal species.

Because no relevant data have been submitted on the metabolism and total residue depletion of nitrofurazone, the Director concludes that he cannot determine the amount of total residue and its depletion with time in the edible products for any of the approved uses of nitrofurazone. Therefore, the first step necessary to estimate probable consumption of residues, which is a required consideration of section 512(d)(2)(A) of the act, has not been fulfilled.

B. *Sufficient Information Has Not Been Provided about the Residues to Determine the Appropriate Testing Required in Laboratory Animals to Estimate the Cumulative Effects of Human Exposure to the Residues (Step 2).* The composition of a drug's residue may vary from the extremes of less than 1 percent parent drug and more than 99 percent metabolites to less than 1 percent metabolites and more than 99 percent parent drug, though the latter case would be rare. The Food and Drug Administration must assess the toxicity of the total residue because this is what man will be exposed to in food. This assessment would be based ideally on the identification of each of the metabolites in the residue, their synthesis, and subsequent testing of the parent drug and all the metabolites for the cumulative effects of exposure. However, this approach is usually impractical.

Various approaches are available to limit the number of residue compounds for which toxicity testing is required to provide a sufficiently conservative estimate of the toxicity of the total residue. However, Norwich has not presented any quantitative or qualitative information about the total residues of nitrofurazone in the edible tissues or milk.

Therefore, the Director concludes that not only is the toxicological testing provided for nitrofurazone inadequate, but he is unable to determine whether testing of metabolites in addition to the parent drug is required to determine the safe level of human exposure to residues in food. Step 2 has not been fulfilled; therefore, the requirements for step 3 cannot be fully determined.

C. *Adequate Dose-Response Data in Test Animals to Determine the Cumulative Effects of Dietary Exposure to the Residue Compounds Has Not Been Provided; Therefore, the Maximum Acceptable Concentration of Residues in Food Cannot Be Established Using Appropriate Safety Factors to Assure that Human Exposure to Residues in the Total Diet Does Not Exceed a Level Deemed Safe (Step 3).* As discussed under section I of this notice, the Director has concluded that adequate testing of the parent drug, nitrofurazone, has not been provided. Also, under steps 1 and 2 (paragraphs A and B of this section), the Director concluded that adequate information has not been provided about the residues to determine if toxicity testing of residue compounds in addition to the parent drug is required to estimate conservatively the cumulative effects of exposure to the residues.

The Director concludes that without such essential information about the residues and adequate toxicity testing data, he cannot, in light of the new evidence, determine if nitrofurazone requires regulation as a carcinogenic animal drug or, alternatively, to reasonably estimate a maximum acceptable level of residue in food that can be deemed safe. Therefore, he concludes that adequate information has not been provided to assess the cumulative effects of exposure and to determine the appropriate safety factors required in section 512(d)(2)(B) and (C) of the act.

D. *Sufficient Information about the Residues Has Not Been Provided to Establish the Conditions Necessary for the Analytical Method Used to Control Residues (Step 4).* The evaluation of an analytical method for residues first requires a determination of the tissues to be analyzed, the compound to be measured, and the lower limit of measurement that must be achieved to control total residues adequately. Only then, after selection of these parameters, is it meaningful to consider whether the method proposed by the sponsor meets other characteristic necessary for a practical reliable method.

1. Determination of the target tissue: The most practical approach to monitoring and controlling residues in the field is dependent on the selection of that edible tissue in which residues require the longest time to deplete to a "safe concentration." Therefore, by monitoring this tissue to assure that residues are below a "safe concentration," it can be assured that all residues in the carcass are below "safe concentrations." In lieu of this determination (identification of the target tissue), all edible tissues, including milk where applicable, must be monitored. This would place an additional burden on public funds for monitoring.

The target tissue is readily identified if the requirements of step 1 of the evaluation process are met. Norwich has failed to meet these requirements for any of the approved uses of nitrofurazone.

2. Determination of the compound (marker) which can be measured by the analytical method to assure control of total residues: Because the radiolabeled drug approach used initially to estimate total residues is not applicable to regulatory use, an alternative approach must be developed to assure control of total residues after drug approval. This is achieved by selecting an appropriate residue compound, which may be the parent drug or one or more metabolites, in such a way that its measurement serves as a valid indicator that when the marker compound is below a certain level in the target tissue, all residues in edible tissues are at safe levels. This step is esential to determine if the method of analysis for regulatory use is adequate. To determine a valid marker, adequate information, e.g., information derived from conducting an adequate metabolism study, is required that relates the proportionate amount of each of the residues to the total residue and their change in quantitative relationships with time. The marker must be a known proportion of the total residue when the total residue depletes to the "safe concentration" in the target tissue. It also must be structurally identified so that an adequate and specific method of analysis can be developed for its determination.

The regulatory method proposed by Norwich to control residues uses the parent drug, nitrofurazone, as a marker in each of the major edible tissues. Norwich has failed to submit substantiating evidence to show that the measurement of nitrofurazone is adequate to control total residues of potential concern or, alternatively, to show that all other residues are safe and do not need to be controlled.

3. Determination of the lower limit of measurement required for the analytical method: Establishment of the lower limit of measurement required for the assay begins with the availability of adequate toxicological testing to determine the cumulative effects of exposure. As described in section I of this notice and in steps 2 and 3 of this section, the sponsors have not provided adequate testing. Therefore, an estimate of the acceptable level of exposure to total residues in food cannot be determined.

Even if adequate testing data were available to estimate the acceptable level of exposure to total residues in food, Norwich has not provided adequate information about a marker compound to determine the lower limit of measurement required for the assay.

As previously stated, Norwich has proposed that the measurement of nitrofurazone is adequate to assure control of residues, but it has not provided any information to show whether nitrofurazone may be, for example, 80 percent of the total residue or 10 percent of the total residue. Therefore, it is not possible to determine at what concentration the marker must be measured to assure that total residues are at a "safe concentration."

After making the adjustment for the percent of total residue that the marker represents, one further adjustment is necessary to determine the lower limit of measurement required for the assay. This last adjustment takes into account the degree to which the method of analysis recovers the residue from the sample. Minimum standards for recovery for the acceptance of the method take into account that 100 percent recovery is rarely achieved and that a combined evaluation of the precision and accuracy for measurement of the marker must be considered.

Norwich is aware of the principles just described for the selection of a target tissue, marker compound, and the adjustments required to establish condi-

tions for the assay; this is evidenced by Norwich's citation of a paper prepared by two FDA scientists in Norwich's submission for furazolidone (NF–180) to FDA on November 11, 1975. The cited paper by M. K. Perez and N. E. Weber, entitled "Requirements for Metabolic Studies—Impact on Food Safety Regulation" was presented at the Gordon Research Conference on Drug Metabolism, June 23–27, 1975.

The Director concludes that he cannot make an assessment of the toxicity, including the carcinogenic potential and potency, of nitrofurazone without adequate testing data; he cannot conclude that nitrofurazone serves as a valid marker for control of total residues of toxicological interest; and therefore he cannot without adequate supporting data determine the conditions necessary for the analytical method used to control residues. The requirements of step 4 have not been fulfilled. He concludes that the new evidence on nitrofurazone (NF–7) and furazolidone (NF–180) leads to a determination that the methods of analysis for residues of nitrofurazone (NF–7) are not shown to be adequate.

E. *Practicable Methods of Analysis are Not Available to Assure that Total Residues in the Daily Diet of Humans Do Not Exceed a Level Deemed Safe (Step 5).* Section 514.1(b)(7) (21 CFR 514.1 (b)(7)) requires the sponsor of a new animal drug application to provide a practicable method of analysis to be used for the control of residues of its product. A "practicable" method is defined by FDA as one that reliably measures the compound throughout all residue levels of interest, and also is sufficiently rapidly and reasonable in terms of the expertise and equipment required to be applicable for use in the surveillance and control of residues. "Reliability" is a combination of accuracy, precision, and specificity. Accuracy and precision are quantitative terms. "Accuracy" is the measure of deviation from the true value, and "precision" refers to the variability of measurement in replications either by one analyst or within a laboratory or between laboratories. A method may provide reliable measurement if the measurements are not too variable even though it measures less than or more than 100 percent of the true value. Therefore, accuracy and precision must be considered together.

"Specificity" is a qualitative term. The measurements obtained by the method must reflect only the compound of interest. Because the method will be used on samples from animals for which exposure history is unknown, the techniques used in the method must be adequate to assure not only the integrity of the measurements, but also the identity of the residue to the exclusion of other interfering substances. Therefore, when the quantitative measurements are made by techniques that are not inherently specific, a confirmatory test is required as part of the official method to assure the identity of the residue.

The submitted methodology for nitrofurazone is based on chromatographic separation and fluorometric determination; both are nonspecific techniques. The uses of nitrofurazone were approved before that time when regulatory methods of analysis for residues were required. In 1960, a method of analysis for tissues capable of measuring nitrofurazone as low as 1 part per million (ppm) was published in Analytical Chemistry, 32(12):1676–1678. In 1962, FDA was provided with a method of analysis for nitrofurazone in milk; the method was claimed to be capable of measuring nitrofurazone as low as 0.01 ppm. This method was subsequently published in the Journal of Agricultural and Food Chemistry, 12:121, 1964. About the same time, revised methodology for tissue residues was also submitted to FDA; the revised methodology was claimed to be capable of measuring nitrofurazone as low as 0.1 ppm in tissue.

None of these methods have been subjected to validation trials by FDA. The data submitted in support of these methods only include studies of the recovery of nitrofurazone from samples to which known amounts of the drug have been added, i.e., spiked samples. No evidence has been submitted to show that the methods will satisfactorily recover nitrofurazone when the residue is naturally incurred through drug treatment, i.e., dosed samples.

After FDA received reports that nitrofurans had induced tumors in female rats, the drug sponsors were advised in 1969 and officially informed in a conference held January 4, 1971, that the residue methods were no longer deemed adequate. They were told that methods capable of measuring at least 2 ppb of the drug in edible tissues and at least 0.2 ppb of the drug in milk were required. In response to the notices of opportunity for hearing, Norwich and other sponsors submitted newly developed methods of analysis.

For the measurement of tissue residues, Norwich concentrated primarily on the method for furazolidone (NF–180) in the belief that this method could be readily adopted for the measurement of tissue residues for the other nitrofurans.

A discussion of the results on the evaluation of the furazolidone (NF–180) method for tissue analysis (including swine liver, kidney, muscle and fat and poultry liver, kidney, muscle and skin with adhering fat) is presented in the May 13, 1976 FEDERAL REGISTER notice for furazolidone. In that notice the Director concluded that the methods for edible tissue were not shown to be adequate.

Although the drug sponsors have submitted far more information to support the adequacy of the methods of analysis for furazolidone (NF–180) than they have submitted on methods of analysis for nitrofurazone, the sponsors have in the intervening years submitted some progress reports on the development of analytical methods for nitrofurazone.

In 1971, three different progress reports were submitted on the analysis of nitrofurazone in chicken tissues.

1. Project No. 475–17 Interim Report, "Chemical Method for Determination of Concentrations of Nitrofurazone in Chicken Liver at 2 ppb." Submitted to FDA with cover letter dated April 29, 1971.

The method presented was based on an ultraviolet densitometric measurement similar to the original method submitted for furazolidone (NF–180), which was rejected by FDA after a desk review of the supporting data. In the nitrofurazone submission, no recovery data were submitted. The results of analysis of five control chicken liver samples were submitted and compared to results obtained on three samples, each fortified with 2 ppb and 4 ppb of nitrofurazone. The measured responses for the samples fortified at the same level were quite variable, though it appeared that the method could perhaps distinguish between fortified and nonfortified samples. In a conference on May 4, 1971, Norwich was advised that the data were too meager for reasonable evaluation.

2. By letter dated May 17, 1971, Norwich provided data on the recovery of nitrofurazone from chicken liver, muscle, and skin-with-fat samples. The same ultraviolet densitometric method described in study 1 above was used. Norwich reported the following recoveries and standard deviations from tissues fortified with 2 ppb of nitrofurazone: liver (49±15%, muscle (47±14%), and skin-with-fat (39±5%). These recoveries were presumably based on the use of an internal standard where corrections were made for aliquot losses and losses in partitioning between solvents; so, they appear higher than the actual recoveries. The firm submitted data for samples fortified with nitrofurazone at 2 ppb, 4 ppb, and 6 ppb. These comparative data showed that the method could not reliably distinguish between the presence of 2 ppb and 4 ppb of nitrofurazone in liver tissue. Also, the single skin-with-fat sample fortified with 6 ppb nitrofurazone produced a lower reading than the one sample fortified with 4 ppb of nitrofurazone.

3. On September 17, 1971, Norwich submitted data on the analysis of chicken kidney for nitrofurazone using the same ultraviolet densitometric method used in studies 1 and 2 described above. Data were provided on peak areas, but sufficient data were not provided to calculate recoveries. The analytical response for the control tissues was as high as 66 percent of the response obtained with tissues fortified with 2 ppb of nitrofurazone.

After 1971, Norwich apparently abandoned the ultraviolet densitometric approach and concentrated on the method of analysis for furazolidone (NF–180) that was subjected to a method trial by FDA and USDA in 1972. They also explored the use of new instrumentation (programmed multiple development thin layer chromatography (PMD–TLC)) and mechanization of spotting thin layer chromatography plates.

By letter dated September 6, 1974, Norwich submitted a status report to FDA on the analysis of nitrofurazone in chicken and swine tissue using the PMD–TLC method. This report included the results of analyses of samples of chicken mus-

cle, kidney, and skin-with-fat and swine muscle, fat, liver, and kidney fortified at 2 ppb, 4 ppb, and 6 ppb with nitrofurazone. No data were presented on the analysis of chicken liver. Recoveries for the samples fortified with 2 ppb of nitrofurazone were calculated by FDA as follows: chicken muscle (112–141%), chicken kidney (84–103%), chicken skin-with-fat (46–76% with the exclusion of one of the four samples where there was an interfering fluorescent band), swine liver (108–142%), swine muscle (82–127%), swine kidney (81–117%), and swine fat (67–116% with the exclusion of one of four samples as an outlier).

Due to the within-laboratory variability in the results, FDA chemists rejected the data as not adequate to warrant interlaboratory validation of the method. It appeared unlikely that the method, if subjected to validation trials, would prove reliable for measurement of nitrofurazone at 2 ppb. Also, as stated previously, until adequate data are presented as described in steps 1 through 4 of the safety evaluation process, the conditions required of a regulatory assay cannot be determined and, therefore, a validation trial is of no value at this stage.

No supporting data have been presented on a method of analysis for edible carcass tissue of food animals other than chickens and swine for which nitrofurazone is approved.

In response to the March 31, 1971 notice of opportunity for hearing on nitrofurazone, Pharm-House Inc. submitted a method of analysis for nitrofurazone in milk; it claimed the method could detect nitrofurazone as low as 2 ppb. The method was based on colorimetric determination of a phenylydrazone derivative of nitrofuzazone. This method was based on essentially the same derivatization technique and measurement as the method submitted by Norwich in 1962, but the Pharm-House method used a different chromatographic cleanup of the extract and concentration of the colored analyte before the colorimetric determination of the nitrofurazone derivative. No recovery data were submitted and a satisfactory standard curve was not presented. Control milk samples showed a response 50 percent of that measured for samples fortified with 2 ppb of nitrofurazone. Only two samples at each fortification level were reported; that is an insufficient number. From the data submitted it is doubtful that the method could provide reliable measurement of nitrofurazone at 2 ppb even if this level were found adequate.

None of the submitted methods of analysis for nitrofurazone in edible carcass tissue or milk are inherently specific, and no confirmatory tests have been provided by the sponsors that could be used to confirm the identity of any residue found by the proposed assay methods.

The Director concludes that the sponsors have not shown that adequate practicable methods of analysis for residues of nitrofurazone in edible carcass tissue and milk are available. Appropriate data have not been provided to determine if nitrofurazone can be used as a marker to control total residues in edible products, nor to determine the lower limit of measurement required for a method to assure that total residues do not exceed safe levels. The Director concludes that the methods proposed by the sponsors for the determination of nitrofurazone are not quantitative and are not adequately specific at the levels of measurement that are likely to be necessary based on the tumorigenic property of the drug. Therefore, the methods proposed are not quantitatively or qualitatively reliable practicable methods for regulatory use, even if it were established that measurement of nitrofurazone was adequate to control total residues. Therefore, step 5 and the requirements of section 512 (b) (7) of the act have not been met.

F. *Practical Conditions of Use Cannot be Prescribed on the Label of Products to Assure Safe Use (Step 6).* Section 512 (b) (8) of the act requires that conditions of use assure that there are no unsafe residues, and section 512(d) (2) (D) of the act adds a further requirement that these conditions be such that they are "reasonably certain to be followed in practice."

It is required by FDA that residue depletion studies be conducted for each drug use under simulated field use conditions to develop data for establishing the withdrawal period. A withdrawal period is the time following drug treatment when, because of residues, a treated animal cannot be slaughtered for food and its milk must be discarded. A withdrawal period must be sufficiently long to assure adequate residue depletion, but it cannot be so long that it is impractical and unlikely to be followed in practice. An adequate analytical method for residues, preferably the same method that will be approved for regulatory use, must be used to measure the residues in the depletion studies.

Only two reports have been submitted by the sponsors of nitrofurazone on residue depletion, and both studies measured only the presence of the parent drug nitrofurazone:

1. Final Report Problem 466.03.23, Phase II and 466.27.23, "Tissue Residues in Swine from Furacin Water Mix," April 19, 1967, NADA 9–415, Vol. 3. From cover letter to FDA, dated January 8, 1971.

Swine were administered nitrofurazone at 0.011 percent in drinking water for 14 days. No nitrofurazone was detected in the edible tissues (liver, kidney, fat, or muscle) at zero day's withdrawal by a chemical method of analysis that Norwich claimed was capable of detecting nitrofurazone as low as 0.1 ppb.

2. Dr. J. L. Mertz's Memorandum to Dr. J. P. Heotis, "Tissue Residue Withdrawal Studies for Nitrofurazone (Amifur) in Chickens Fed Nitrofurazone at 150 Grams Per Ton of Feed for 14 Days," November 8, 1971, NADA 9–415, Vol. 3.

Chickens were fed nitrofurazone at 150 grams per ton in feed for 14 days. Animals were slaughtered at 3, 4, and 5 days after withdrawal from medication. Edible tissues were examined using the TLC-ultraviolet based methodology, which the sponsor claimed could detect nitrofurazone as low as 2 ppb. However, FDA had not accepted this method because of the variability in the recovery data submitted. Only kidney samples were analyzed after 3 days' withdrawal. Samples of kidney, liver, muscle, and skin with fat were analyzed at 4 and 5 days' withdrawal. One of four kidney samples was reported to contain 2 ppb of nitrofurazone at 3 days' withdrawal. One of two kidney samples was reported to contain nitrofurazone above 2 ppb after 5 days' withdrawal. All other samples analyzed at the various withdrawal times were reported to contain no detectable nitrofurazone.

No residue depletion data have been provided for the other food-producing animals for which the use of nitrofurazone is approved, i.e., poultry other than chickens and large animals other than swine.

Nitrofurazone was originally approved for the treatment of mastitis in 1949, including both lactating and dry cow treatment. However, in 1963, the sponsor revised the labeling to limit its use to dry cow treatment only. No residue depletion studies have been submitted to demonstrate whether this dry cow treatment results in residues in mulk after the treated animals freshen or whether the treatment would result in residues in the edible carcass tissues that would be of concern in the event the animal was culled from the dairy herd for slaughter.

The Director concludes that withdrawal periods are likely to be necessary for the safe use of nitrofurazone. The currently labeled withdrawal period for chickens, turkeys, and swine voluntarily used now by the sponsors is 5 days. The Director concludes that data to support this withdrawal period or any practical withdrawal period for any of the uses have not been provided because:

a. No data for any of the uses have been submitted on the amount of total residue (parent drug and metabolites) after drug treatment and the depletion with time of total residues of concern.

b. No data have been provided to show that only the parent drug compound is of concern in evaluating the safety of the residue.

c. No data have been provided to show whether the measurement of nitrofurazone in edible products can be used to control total residues and, if so, at what level its measurement is required to assure that all residues are below concentrations deemed safe.

d. The residue depletion studies submitted for chickens and swine are not shown to be adequate even if nitrofurazone could be shown to be an adequate marker for total residues. In the swine study the method of analysis would not be sufficiently sensitive based on the known tumorigenic properties of the drug. Similarly, the method use in the chicken study, even if it could be shown that nitrofurazone measurement at 2 ppb was adequately sensitive, is not acceptable because the supporting data on the

method are not adequate to assure the reliability of results.

e. No depletion data have been submitted for uses of nitrofurazone in poultry other than chickens and in large animals other than swine, or for safe use of nitrofurazone as a dry cow treatment for mastitis.

The requirements of step 6 and section 512(b)(8) and (d)(2)(D) of the act have not been fulfilled.

Therefore, the Director is hereby withdrawing the notice of opportunity for hearing issued March 31, 1971, proposing to withdraw approval of NADA's providing for the use of nitrofurazone (NF-7) in food-producing animals. Under section 512(e)(1)(B) of the act (21 U.S.C. 360b(e)(1)(B)), the Director proposes to withdraw approval of certain existing NADA's providing for the use of nitrofurazone in food-producing animals on the grounds that such use violates section 512 of the act in that such use has not been shown to be safe.

This notice applies to all outstanding NADA's for the use of nitrofurazone in food-producing animals. All such applications known to FDA are listed below by NADA number and by each applicant's name and last known address in FDA records. Copies of this notice are being forwarded by registered or certified mail to each of the known applicants. Notice to other applicants or holders of NADA's whose identity is not known to FDA is given by this publication.

6-395, 7-801, 8-324, 8-410, 8-529, 8-784, 10-741, Hess & Clark, Division of Rhodia, Inc., 7th and Orange St., Ashland, OH 44805.
6-475, 8-129, 9-415, Eaton Laboratories, Division of Morton-Norwich Products, Inc., P.O. Box 191, Norwich, NY 13815.
6-649, E. F. Drew & Co., Farm Feed Division, Boonton, NH 07005.
9-013, Nopco Chemical Co., 60 Park Place, Newark, NJ 07102.

Any holder of an approved application that elects to request an opportunity for hearing pursuant to section 512(e)(1) of the act and § 514.200 of Chapter I of Title 21 of the Code of Federal Regulations must file with the Hearing Clerk, Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, MD 20852, a written appearance requesting such hearing by September 16, 1976, giving the reasons why approval of the application should not be withdrawn, and providing a well-organized and full-factual analysis of the scientific and other investigational data such holder is prepared to prove in suppport of its opposition to the Director's proposal. A request for hearing may not rest upon mere allegations or denials but must set forth specific facts showing that there is one or more genuine and substantial issue(s) of fact requiring a hearing. Responses to this notice and copies of published literature cited in this notice may be seen at the office of the Hearing Clerk, Food and Drug Administration, during regular business hours, Monday through Friday.

If a hearing is requested and is justified by any holder's response to this notice of opportunity for hearing, the issues will be defined, an Administrative Law Judge will be assigned, and a written notice of the time and place at which the hearing will commence will be issued as soon as practicable.

Any hearing on withdrawal of approval of the foregoing NADA's will be open to the public. If, however, the Director finds that portions of the applications that serve as a basis for such a hearing contain information concerning a method or process that is entitled to protection as a trade secret, the part of the hearing involving such portions will not be public, unless the respondent so specifies.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (sec. 512, 82 Stat. 343-351 (21 U.S.C. 360b) and under authority delegated to the Commissioner (21 CFR 5.1) and redelegated to the Director of the Bureau of Veterinary Medicine (21 CFR 5.29) (recodification published in the FEDERAL REGISTER of June 16, 1976 (41 FR 24262)).

A discussion of the economic and environmental impact of this action is set forth in the notice of proposed rule making on nitrofurazone published elsewhere in this issue of the FEDERAL REGISTER.

It is hereby certified that the economic and inflation effects of this proposal have been carefully evaluated in accordance with Executive Order 11821.

Dated: August 6, 1976.

C. D. VAN HOUWELING,
Director, Bureau of Veterinary Medicine.

[FR Doc.76 23622 Filed 8-10-76;11:19 am]

[Docket No. 76N-0233]

HESS & CLARK, ET AL.

Nihydrazone (NF-64); Opportunity for Hearing on Proposal To Withdraw Approval of Certain New Animal Drug Applications

The Director of the Bureau of Veterinary Medicine of the Food and Drug Administration (FDA) is issuing a notice of opportunity for hearing on a proposal to withdraw approval of approved new animal drug applications (NADA's) Nos. 13-195 and 13-200 for the use of nihydrazone (NF-64) in food-producing animals in accordance with section 512(e)(1)(B) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b(e)(1)(B)) (hereinafter, "the act") on the grounds that new evidence not available when the applications were approved, evaluated together with the evidence available at the time of approval, shows that nihydrazone is not shown to be safe for use under either the approved or currently labeled conditions of use. Holders of approved NADA's have until September 16, 1976 to submit requests for a hearing in accordance with § 514.200 (21 CFR 514.200).

In FR Doc. 76-23620, appearing elsewhere in this issue of the FEDERAL REGISTER, the Director is issuing a notice of proposed rule making to revoke the appropriate provisions of sections affected by the proposed withdrawal.

Nihydrazone (NF-64) is a member of a class of chemicals called "nitrofurans," which are used at low levels in animal feed as antibacterial and antiprotozoan agents to increase resistance to disease, thereby assisting growth. Nitrofurans also have prescribed therapeutic uses. In addition to nihydrazone (NF-64), which is the subject of this notice, there are three other nitrofurans that are used in food-producing animals and are or have been subject to agency action: nitrofurazone (NF-7) and furaltadone (NF-260), which are subjects of companion notice in this issue of the FEDERAL REGISTER; and furazolidone (NF-180), which was the subject of notices in the FEDERAL REGISTER of May 13, 1976 (41 FR 19906-19907). A fifth nitrofuran, furamazone (NF-84), is not at this time the subject of contemplated agency action for withdrawal.

Nihydrazone (NF-64) was first approved in 1963. It is currently approved for use in medicated broiler and replacement chicken feeds for the prevention of chronic respiratory disease (air sac infection), pullorum disease, fowl typhoid, paratyphoid (salmonellosis), histomoniasis (blackhead) and coccidiosis caused by *Eimeria tenella*, *E. necatrix*, *E. maxima*, and *E. brunetti*.

The original applications were approved without a requirement that the drug treatment be discontinued for a specified period of time before slaughter of the animals. However the sponsors, Norwich Pharmacal Co., Division of Morton-Norwich Products, Inc., and Hess & Clark, currently a Division of Rhodia, Inc., voluntarily revised the labels in January 1971 to require that chickens be withdrawn from nihydrazone (NF-64) 4 days before slaughter.

The Food and Drug Administration issued a notice of opportunity for hearing published in the FEDERAL REGISTER of March 31, 1971 (36 FR 5927) proposing to withdraw approval of NADA's for nihydrazone (NF-64) on grounds that:

> Information available to the Commissioner establishes that the drugs, when administered to laboratory animals, have been shown to produce tumors. The drugs are, therefore, not considered to be safe for use in the absence of appropriately sensitive methods of analysis to establish their absence in food derived from treated animals.

Similar notices were published for nitrofurazone (NF-7) in the FEDERAL REGISTER of March 31, 1971 (36 FR 5926) and for furazolidone (NF-180) and furaltadone (NF-260) on August 4, 1971 (36 FR 14343). The history leading to the publication of these 1971 notices is discussed in the May 13, 1976 notice for furazolidone (NF-180).

Norwich and Hess & Clark responded to the March 31, 1971 notice concerning nihydrazone (NF-64) by filing requests for a hearing. Norwich assured FDA that it had adequate methods of analysis to determine residues and adequate data to show that under the currently labeled conditions of use the drugs were safe. In addition, Norwich set forth a hypothesis that nitrofurans were not primary

tumorgens but stimulated spontaneous tumor growth by disrupting the normal hormone balance.

Norwich has continued to submit data pertaining to the use of nihydrazone (NF–64) in food-producing animals. The Food and Drug Administration has determined that the new evidence, evaluated together with the evidence available when the approvals were granted, fails to demonstrate that either the approved or currently labeled uses of nihydrazone are shown to be safe. The Director is withdrawing the March 31, 1971 notice of opportunity for hearing concerning nihydrazone (NF–64), and is issuing this new notice, which presents in detail all the evidence now available to him, and the basis on which he proposes to withdraw approval of all uses of nihydrazone (NF–64) in food-producing animals in accordance with section 512(e)(1)(B) of the act.

Section 512(e)(1)(B) of the act requires that FDA withdraw approval of an NADA when "new evidence * * * evaluated together with the evidence available * * * when the application was approved, shows that such drug is not shown to be safe * * *." Under this provision, the new evidence and the evidence submitted at the time the application was approved must be evaluated to determine whether all of the requirements for safety contained in section 512 of the act continue to be fulfilled.

The Determination of "Safety" in Section 512

Section 512 of the act requires that the use(s) of a new drug in food-producing animals must be supported by adequate data supplied by the sponsor in applications that include, among other things, demonstration that the drug's use will not result in unsafe residues in human food.

Under section 512(b) of the act, whenever a drug's use may result in residues of the drug in food, an application must include not only full reports of investigations to show that the use of the drug is safe, but also a description of practicable methods for monitoring food to assure there are no unsafe residues in human food attributable to the drug's use, and demonstration that the conditions of use are adequate to assure there are no unsafe residues.

Under section 512(d)(2) of the act, FDA is required, in the evaluation of the supporting safety data, to consider, among other things, the following:

1. The probable consumption of such drug and of any substance formed in or on food because of the use of such drug (i.e., probable human consumption of residues, including the parent drug and metabolites).

2. The cumulative effect of such drug on man or animals, taking into account any chemically or pharmacologically related substance (i.e., toxicological effects of the compounds comprising the residues).

3. Safety factors that, in the opinion of experts qualified by scientific training and experience to evaluate the safety of such drugs, are appropriate for the use of animal experimentation data (i.e., establishing "safe" levels of residues using appropriate safety factors to extrapolate animal data on cumulative effects to humans).

Under section 512(d)(1)(H) of the act, additional requirements must be met if the drug is a carcinogen.

The determination of safety within the meaning of section 512 of the act for a drug whose use may result in residues in the edible products of the treated animal, whether or not the drug is a carcinogen, requires a multistep process. These steps form an orderly procedure by which information is derived to satisfy each of the three considerations stated above.

Step 1. Assessment of the total residues in edible products and the depletion of those residues with time following cessation of drug treatment in order to determine probable human consumption (section 512(b)(1) and (d)(2)(A) of the act).

Step 2. Assessment of information about the residues to determine the appropriate testing in laboratory animals required to estimate the cumulative effects of human exposure to the residues (section 512(b)(1) and (d)(2)(A) of the act).

Step 3. Acquisition of dose-response data in test animals to determine the cumulative effects of dietary exposure to the residue compounds from which the maximum acceptable concentration of residues in food can be established using appropriate safety factors to assure that human exposure to residues in the total diet does not exceed a level deemed safe (section 512(b)(1) and (d)(2)(B) and (C) of the act.) In the case of a carcinogenic drug, a conservative statistical extrapolation of the tumor data is used to estimate a level of residue exposure that could be considered insignificant and poses essentially no risk of cancer and operationally defines "no residue" for purposes of computing the residue assay requirements under section 512(d)(1)(H) of the act.

Step 4. Acquisition of information about the residues to (1) determine the tissue (called the target tissue) from which residues require the longest time to deplete to safe levels and which, therefore, must be monitored by the regulatory assay (step 5), and (2) determine the compound (called marker) that is to be measured in the regulatory assay so that by its measurement there is assurance that no unsafe resides occur from the use of the drug (section 512(b)(7) of the act).

Step 5. Development of a practicable method of analysis that can measure the marker in the target tissue at a concentration that assures that total residues in the daily diet of humans do not exceed the level established in step 3 as safe (section 512(b)(7) of the act).

Step 6. Demonstration that practical conditions of use can be prescribed on the label of the product to assure there will be no unsafe residues in the food products from treated animals (section 512(b)(8) and (d)(2)(D) of the act).

Each of the steps involves principles that are essential for a determination of food safety and compliance with the requirements of the act to assure there are no unsafe residues in human food from the animal drug use. Each step builds upon the preceding step. Therefore, failure to accomplishment any one of the six steps in whole or in part for any drug ordinarily will preclude a sponsor's fulfilling its obligation under the act to determine probable consumption of residues and cumulative effects of exposure, and to establish safe conditions of use with appropriate regulatory methods of analysis to control residues when deemed necessary.

New evidence concerning nihydrazone (NF–64) not available when the applications were approved includes, among other things, a demonstration that nihydrazone induces mammary tumors in female rats. The Director has evaluated all the data now available, including the new data received since 1963 when uses for nihydrazone were first approved and the data available to FDA at the time of approval, and has concluded that the approval of NADA's for nihydrazone must be withdrawn under section 512(e)(1)(B) of the act because:

1. Adequate investigations have not been provided to show that the uses of nihydrazone are safe, as required by section 512(b)(1) of the act. Adequate information has not been provided on the probable consumption of residues, and the cumulative effects of exposure have not been determined by appropriate tests, as required by section 512(d)(2)(A) and (B) of the act. Therefore, "safe" levels of residue cannot be determined using appropriate safety factors, as required by section 512(d)(2)(C) of the act.

2. Adequate, reliable, and practicable methods of analysis are not available for monitoring food to assure there are no unsafe residues in edible tissues that are attributable to the use of nihydrazone, as required by section 512(b)(7) of the act.

3. Practical conditions of use cannot be specified in the labeling to assure there are no unsafe residues attributable to the use of nihydrazone, as required by section 512(b)(8) and (d)(2)(D) of the act.

The data on which these conclusions are based are presented in the succeeding sections of this notice. The Director first describes all the relevant new evidence bearing on the determination of safety for the uses of nihydrazone. Then he evaluates all the evidence before him using the six-step procedure necessary to satisfy a human safety determination for the use of nihydrazone (NF–64) and to meet the considerations required in section 512 of the act.

I. New Evidence

In October 1966 and in January 1967, Norwich submitted reports of the effect in rats of feeding diets containing nitrofurans for 45 weeks. These studies included tests on nihydrazone (NF–64) and furazolidone (NF–180). In these studies it was shown that both nihydrazone (NF–64) and furazolidone (NF–180) in-

duced mammary tumors of similar type in female rats.

Subsequently, more extensive, longer term testing of furazolidone (NF–180) was conducted, but further testing of the carcinogenic potential of nihydrazone (NF–64) has not been undertaken. The more extensive testing of furazolidone (NF–180), as described in the May 13, 1976 FEDERAL REGISTER, shows that the drug induces cancer in both rats and mice and that the tumors and cancers were not limited to the mammary gland or the endocrine tissues of female rats.

The Director is including in this notice information available on the toxicological properties of furazolidone (NF–180) as well as nihydrazone (NF–64) because of the structural similarities of the drugs and their similar biological properties, which have been observed in the limited comparative data available. The new evidence showing that furazolidone (NF–180) is a carcinogen has a significant bearing on the Director's conclusions that the uses of nihydrazone (NF–64) are not shown to be safe.

Although it is appropriate to consider data on furazolidone (NF–180) to judge the adequacy of existing data to support the safe use of nihydrazone (NF–64) in food-producing animals, these data are not a substitute for data on nihydrazone (NF–64) that are required by the statute. Basic data are required for each drug, recognizing that slight differences in chemical structure can result in significant differences in metabolism, residues, and toxicity. Although comparative data on the biochemical and toxicological properties of related drugs may be useful and appropriate in making decisions about the degree of testing required for each drug to show there is no probable hazard, these data cannot be used as a substitute for the basic information necessary on each drug to determine the probable exposure to residues and cumulative effects of exposure.

A. *Carcinogenic Potential of Nihydrazone.* Norwich submitted data in October 1966 and January 1967 on the cumulative effects in rats of feeding diets containing nihydrazone (NF–64) for 45 weeks ("Effects of Long Term Feeding of Various Nitrofurans, October 13, 1966 and January 4, 1967, Project No. 440.07, submitted to FDA as part of a June 2, 1967 drug experience report). These studies include data on testing in female Sprague-Dawley (Holtzman strain) rats and testing in both male and female Carworth Farms (CFE strain) rats.

1. The Sprague-Dawley Female Rat Study: Thirty-five female Sprague-Dawley rats (Holtzman strain) were fed nihydrazone at about 0.06 percent (34 milligrams per kilogram (mg/kg) body weight per day) in their diet for 45 weeks, followed by maintenance on an unmedicated diet for an additional 8 weeks before sacrifice. A comparable control group of female rats was maintained on a nihydrazone-free diet for 53 weeks. At termination of the study the animals were autopsied, and tissues were examined for histopathological lesions.

The most prominent drug-related effect was the induction of tumors in the mammary glands of the female rats. Although rats develop mammary tumors spontaneously as they age, the incidence of spontaneous mammary tumors in rats just over 1 year old, depending upon the strain of rat used, can be quite loww. The nihydrazone-treated female rats had an increased number of mammary tumors, particularly the number of fibroadenomas, as compared with the control rats. The development of tumors included the finding of some mammary adenocarcinoma (cancer).

Norwich reported that no mammary tumors of any type were observed in the 33 control rats examined. It reported from histopathological examination that a total of 11 mammary tumors were found in 9 of the 32 nihydrazone-treated female rats examined. Two of the mammary tumors were reported by Norwich as adenocarcinomas. Norwich did not designate whether these cancers were found in one or two rats.

An Ad Hoc Committee was established to review the pathology data on all the nitrofurans tested in the Sprague-Dawley rat study and the Carworth Farms rat study. One member of the Ad Hoc Committee was the director of the Eppley Institute for Research in Cancer, University of Nebraska. He and his associate pathologists reviewed the pathology slides of the mammary tissues from 32 of the nihydrazone-treated rats provided by Norwich; they submitted a detailed report to FDA. They did not review all the mammry gland slides from the control rats.

Each of the reviewed slides had a rat identification number, and there were several slides for each rat. However, there was no information on the number of tumors in each rat. Therefore, it was not possible, in many cases, for the pathologists to determine which slides represented different tumors in the same rat and which were different sections of a single tumor.

The Eppley pathologists concluded from their review of the nihydrazone-treated female rats that nine of the rats had mammary tumors that were predominantly fibroadenomas and that three of these rats had mammary adenocarcinoma.

Because the Norwich report did not indicate which rats had tumors, it is not possible from the data available to determine if the tumors reported by the Eppley pathologists were from the same rats in which Norwich had reported tumors. The Eppley pathologists reported mammary adenocarcinoma in three of the nihydrazone-treated rats, whereas the Norwich report only indicates the finding of two mammary adenocarcinomas and does not indicate the number of rats with cancer. However, because the Epply pathologists did not review all the mammary gland slides from the control rats, a comparative incidence of mammary cancer cannot be determined from the reports.

A pathologist from the Bureau of Foods of FDA, who was not a member of the Ad Hoc Committee, later reviewed the pathology slides of the mammary tumors from the nihydrazone-treated rats in which the Eppley pathologists had reported mammary tumors. From his examination of the histopathological preparations provided, he reported nine slides of mammary fibroadenoma from nine rats and three slides of mammary adenocarcinoma from two rats. The mammary adenocarcinomas were diagnosed in the same two rats in which the Eppley pathologists had diagnosed mammary adenocarcinoma. However, the Bureau of Foods pathologist diagnosed a fibroadenoma in the third rat, which has been reported by the Eppley pathologsts to have an adenocarcinoma.

The Bureau of Foods pathologist also did not have information indicating which slides were multiple sections of single tumors or which represented different tumors. He did have information on the rat identification number for each set of slides. Because the Bureau of Foods pathologist did not review all the slides in the study, tumor incidence and the comparison of incidence with the control rats cannot be determined from his report. However, his report supports the Eppley pathologists' findings that mammary tumors were found in at least nine of the rats treated with nihydrazone and confirms that mammary adenocarcinoma was found in at least two of the nihydrazone-treated rats.

2. The Carworth Farms Rat Study: Nihydrazone (NF–64) was fed to 20 male and 20 female Carworth Farms (CFE strain) rats at about 0.06 percent in the diet (27 to 32mg/kg body weight per day) for 45 weeks, followed by maintenance on an unmedicated diet for an additional 7 weeks before sacrifice. A comparable control group of rats was maintained on a nihydrazone-free diet for 52 weeks. At termination of the study the animals were autopsied, and the tissues were examined for histopathological lesions.

As in the previous Sprague-Dawley rat study, the most prominent drug-related effect seen was the induction of tumors in the mammary glands of female rats. This included an increase in the number of mammary fibroadenomas and the finding of one rat with mammary adenocarcinoma.

Norwich reported that no tumors were observed in the 15 female control and 18 male control rats examined. Norwich reported from histopathological examination that 7 out of 20 nihydrazone-treated female rats examined had a total of 9 mammary tumors. It reported 1 male rat out of 18 nihydrazone-treated male rats as having a tumor mass. However, it is not known what diagnosis was made from the histopathology for this male rat. Norwich also reported that no mammary adenocarcinomas were found in the nihydrazone-treated female rats.

The Eppley pathologists reviewed the slides of the mammary tissues from the 20 nihydrazone-treated female rats provided by Norwich. They also concluded that seven of the treated female rats had mammary tumors, and they reported that

one of these tumors was a mammary adenocarcinoma.

Slides of the mammary tumors in the nihydrazone-treated female rats reported by the Eppley pathologists were later reviewed by the Bureau of Foods pathologist. He reported mammary tumors in each of the seven female rats. From his examination of the histopathological preparations provided, he reported eight slides of mammary fibroadenoma and one slide of mammary adenocarcinoma. The adenocarcinoma was in the same rat in which the Eppley pathologists had reported a mammary adenocarcinoma.

Food and Drug Administration scientists recognize the there are uncertainties and limitations in the examination of tumors to determine if they have potential for malignancy. For example, in the early stages of a mammary tumor growth, the mass may be chiefly adenomatous. As the growth continues, the supporting white fibrous connective tissue may also begin to proliferate, resulting in a fibroadenoma. Later, the adenomatous part of the neoplastic mass may entirely disappear, leaving a fibroma. Finally, a part of the adenoma of fibroadenoma may develop malignant tendencies, and that portion may then become an adenocarcinoma. All four tumor types, i.e., adenoma, fibroadenoma, fibroma, and adenocarcinoma may be present in the same tumor mass. A small focus of adenocarcinoma in the early stage of a malignant tumor may be overlooked in the tumor growth unless adequate multiple sections of the tumor are made.

Also, unless great care is exercised in the evaluation of the spread of an adenocarcinoma of the mammary gland, the metastasis along the lymphatic routes passing through, under, or near other mammary glands may be considered to be separate primary adenocarcinomas rather than a single primary growth with multiple metastases.

The reports from Norwich indicate that nihydrazone (NF–64) caused an induction of mammary tumors in female rats just over 1 year old and that there is an indication of an increase in the number of mammary adenocarcinomas compared with control rats.

Food and Drug Administration scientists conclude that both studies show that nihydrazone is tumorigenic in rats and that the data rose questions about the carcinogenic potential and poency of nihydrazone. They conclude that neither of the studies is adequate to assess the cumulative effects of exposure to nihydrazone and its carcinogenic potential and potency because the studies were not of sufficient duration and the number of animals used was not sufficient. In the Sprague-Dawley rat study only female animals were used; so it is not possible to determine whether nihydrazone might have produced effects in the male rats. In the Carworth Farms rat study, the number of males was too small to determine with any confidence that nihydrazone did not have an adverse effect. In both studies, drug-treatment was for only 45 weeks and included only one dose level. In spite of the weaknesses in the reports available, it is evident that a 45-week treatment was sufficient to induce tumors in female rates, and that some of these tumors were mammary cancers. The dose level, frequency, and duration of exposure to a compound are related not only to whether or not cancer is induced but also to the latency period before the expression of cancer. Therefore, the 45-week treatment with nihydrazone is not considered a sufficient period of exposure to determine adequately the cancer potential or potency.

The Director concludes that the available data provide sufficient cause for concern that nihydrazone may be a carcinogen and that adequate life-time testing in appropriate animal species is required to evaluate its carcinogenic potential and potency. The Director concludes that because nihydrazone is a tumorigen, dose-response data in lifetime studies, using several dose levels, are necessary to develop the information required to assess the cumulative effects of exposure and to establish conditions of safe use. Although the demonstration of cancer induction may be made on the basis of testing at only one dose level, it may be possible from the results of this testing to conclude that a drug requires regulation under the provisions of section 512(d)(1)(H) of the act, dose-response data are necessary to provide information from which FDA can establish the operational definition of "no residue" for purposes of determining the required conditions for the regulatory assay for residues of a carcinogenic drug. Regardless of whetheer adequate testing were to show that nihydrazone induced cancer or induced only benign tumors, dose-response information would be required to establish the conditions of safe use.

The Norwich data from these two studies were available at the time FDA issued the March 31, 1971 FEDERAL REGISTER notice of opportunity for hearing on the proposed withdrawal of approvals for nihydrazone (NF–64). In response to the March 1971 notice, Norwich stated that all four nitrofurans (NF–64, NF–7, NF–180, and NF–260) caused an early development of mammary tumors that were of the type normally found in aging rats, and it postulated that the nitrofurans caused these tumors only at high levels of exposure in the female rats because of disruption in the hormonal system. Norwich presented data purporting to show that the hormonal disruption that produced the tumors involved a process not applicable to humans, and that the proposed methods of analysis for control of residues were more than adequate to assure that levels of residues were below any level which might adversely affect humans.

The Norwich hormonal hypothesis is described in detail in the May 13, 1976, FEDERAL REGISTER notice on furazolidone (NF–180). The supporting information provided by Norwich for the hypothesis was primarily limited to data on furazolidone (NF–180) and some very limited comparatative data on nitrofurazone (NF–6). No data were presented on nihydrazone related to a hormonal mechanism of action.

B. *Comparative Data on Nihydrazone and Furazolidone.* Norwich has provided data on furazolidone (NF–180) from the Sprague-Dawley female rat study and the Carworth Farms rat study It has also supplied data on testing of furazolidone (NF–180) in three chronic rat studies and one mouse study; the tests are described in the May 13, 1976 FEDERAL REGISTER notice. The data have a bearing on the Director's proposed action in this notice for nihydrazone (NF–64) and his decision to reject the Norwich hormonal hypothesis for nihydrazone (NF–64) and furazolidone (NF–180). They also have a bearing on his decision to reject the sponsor's claims that adequate studies have been provided for nihydrazone (NF–64) to support a determination that the drug uses are shown to be safe.

In the Sprague-Dawley female rat study and Carworth Farms rat study, furazolidone (NF–180) was also tested in the protocols; it was tested using a dietary level of about 0.1 percent as compared to the nihydrazone (NF–64) dietary level, which was about 0.06 percent.

In the first study, Norwich reported that 14 of the furazolidone-treated female Sprague-Dawley rats out of 32 rats examined had mammary tumors. The total number of rats with tumors reported by Norwich was roughly twice the number of female rats with tumors reported for the nihydrazone-treated rats. However, the tumor multiplicities reflected in the total number of tumors for the furazolidone (NF–180)-treated female rats were better than three times the number of total tumors found in the nihydrazone-treated rates. Norwich reported five adenocarcinomas in the mammary glands of the furazolidone-treated rats and did not specify in how many rats or in which rats these were found. The Eppley pathologists reviewed the mammary tissue slides from 32 furazolidone-treated female rats and reported tumors in 14 of the rats. The Bureau of Foods pathologist later reviewed slides of the mammary tumors from the 14 rats and reported tumors in all of these rats. Both the Eppley and Bureau of Foods pathologists reported only one mammary adenocarcinoma and one mammary squamous cell carcinoma, each in a different rat, from the slides of the furazolidone-treated rats they had examined. In comparison with these furazolidone-treated rats, the Eppley pathologist found mammary adenocarcinoma in three nihydrazone-treated rats, and the Bureau of Foods pathologist confirmed the cancer diagnosis of the Eppley pathologist in two of the three rats.

In the Carworth Farms rat study, 15 of the furazolidone-treated female rats out of 17 examined were reported by Norwich as having mammary tumors. In com-

parison, Norwich had reported that 7 of the nihydrazone (NF–64)-treated female rats out of 20 examined had mammary tumors. The total number of mammary tumors reported by Norwich in the furazolidone (NF–180)-treated female rats was seven times the total number of mammary tumors that it had reported for the nihydrazone (NF–64)-treated female rats.

Based on these comparative data, the Director concludes that furazolidone (NF–180) and nihydrazone (NF–64) qualitatively exhibit similar tumorigenic effects in female rats. Because the dose levels of the two drugs were not the same (nihydrazone at about 0.06 percent in the diet and furazolidone at about 0.1 percent in the diet), and because the studies are not adequate to assess the cumulative effects of exposure and carcinogenic potential and potency, the data are not sufficient for a precise comparison of the tumorigenic potencies. The Director therefore concludes that although the limited data give some indication that nihydrazone may be a less potent tumorigen than furazolidone, adequate testing of nihydrazone in lifetime studies in appropriate animal species with the development of dose-response data from the administration of nihydrazone at several dose levels is required to determine adequately the carcinogenic potential and potency of nihydrazone.

More definitive studies of the carcinogenic potential of furazolidone (NF–180) in male and female rats and mice provided by Norwich are described in the May 13, 1976 FEDERAL REGISTER notice. A summary of the findings from the three chronic rat studies and the mouse study include the following:

1. Furazolidone induced malignanices in both rats and mice.

2. Furazolidone also induced nonmalignant tumors.

3. In most cases, the induced malignant and nonmalignant tumors were found at the same sites.

4. There is some evidence that the induction of benign tumors is related to the induction of, or progression to, malignancies as a function of dose.

5. There are marked sex differences in the tumor responses in the rats. There are also some commonalities in the tumor sites of the male and female rats.

6. Mammary tumors were the predominant effect in the female rats.

7. Nonmammary tumors were induced in the Sprague-Dawley male rats, with no one type singularly significant. In the Fisher rats, however, several specific nonmammary tumors were induced in both male and female rats.

8. The major target site for tumors in the mouse was the lung. There was no evidence of a significant difference between male and female mice in tumor response, and there were no tumorigenic effects in the endocrine tissues.

9. The induction of tumors involved not only increased incidence of animals with tumors but also an increased number of tumors per animal.

The Director has rejected the data supplied by Norwich to support the hormonal hypothesis for furazolidone (NF–180). A discussion of these data and his basis for rejection are also presented in the May 13, 1976 FEDERAL REGISTER notice. The Director also rejects the Norwich reply to the 1971 FEDERAL REGISTER notice on nihydrazone (NF–64), which attempted to explain the tumorigenic effects of nihydrazone primarily on the basis of the furazolidone (NF–180) data relative to the hormonal hypothesis. The Director finds that the new evidence provided by the more extensive chronic testing data for furazolidone (NF–180), which shows that tumors were induced in both male and female rats and mice and were not limited to tumors of the mammary gland or endocrine tissues, is inconsistent with the hormonal hypothesis.

Therefore, the Director concludes that the comparative data available on furazolidone (NF–180) and nihydrazone (NF–64) in the Sprague-Dawley female rat study and the Carworth Farms rat study, together with the data on the four chronic studies of furazolidone (NF–180) in rats and mice, indicate that adequate testing of nihydrazone (NF–64) has not been provided to assess the cumulative effects of exposure necessary for a determination of safety.

II. USES OF NIHYDRAZONE ARE NOT SHOWN TO BE SAFE

To assess the safety of residues using criteria described in section 512(b) of the act and elaborated on in section 512 (d)(2) of the act requires a multistep process that must take into account consideration of the metabolism of the drug, determination of the resulting residues, including parent drug and the metabolites, the depletion of those residues with time after drug treatment, and the cumulative effect of human ingestion of the residues. The six basic steps involved in this evaluation process have been previously described above.

A. *The Amount of Total Residue in the Edible Products and the Depletion of Residues with Time must be Determined (Step 1).* When a drug is administered to an animal, the drug may be altered by the animal into a variety of compounds called metabolites. The process of alteration may be such that little residue of the parent drug remains in the animal tissue, but the amount of metabolites may be substantial. Therefore, the total residue with which FDA is concerned includes both the parent drug and the metabolites.

The nature and amount of each of the residues depend in part upon the metabolism in a particular species. Because there are species differences in metabolism, it is necessary to determine metabolism and residues in each of the species for which the drug is used. Therefore, § 514.1(b)(7)(i) (21 CFR 514.1(b)(7)(i)) includes metabolism and residue depletion studies among those kinds of studies that are used to determine residues.

Exposure to nihydrazone in the diet-induced mammary tumors in female rats. It is not known whether the parent drug was the active agent or whether the responses were due to one or more active metabolites produced as a result of ingestion of the parent drug by the rats. Such lack of knowledge is not unique to nihydrazone; it is typical of many tumorigenic and carcinogenic agents that the mechanism of the induction of tumors or the identification of the active agent is not known. Therefore, when considering the safety of food from nihydrazone-treated animals, all drug-related residues to which humans may be exposed must be assumed to be potentially carcinogenic unless convincing evidence is presented to the contrary. Such evidence consists of not only the identification of the residues that are claimed to be safe but also adequate toxicological studies showing that the levels of each of the residues claimed to be safe are in fact safe. All residues not identified or not shown to be safe must be considered potentially carcinogenic and must be required to be below a level of measurement that can be considered insignificant and to pose essentially no risk of cancer in humans.

The use of chemical assays to determine initially the total residues would require knowledge about what residues occur (parent drug and metabolites) and would require the availability of adequate methods of analysis to measure at the level of interest each one of the residues in the edible tissues. Therefore, this chemical approach is usually not feasible. Instead, a radiolabeled parent drug is used to provide a practical and adequate approach to the initial measurement of total residues in the edible tissues. In this approach, the drug is synthesized with a radioisotope, so that when the drug is administered to the test animal according to label directions, all resulting residues of potential interest can be measured in the edible products. This is accomplished by counting the radioactivity in the tissues and converting these measurements to an estimation of the amount of residue expressed as parent drug equivalents.

By using radiolabeled nihydrazone administered to chickens, Norwich has measured total radioactivity in the tissues. Norwich has provided these data, which include the study of residue deposition in edible tissues as a function of the length of time of drug administration and the depletion of residues with time after cessation of drug treatment. Comparative data were presented with a carbon-14 label (^{14}C) on the first carbon in the side chain at the 2-position of the furan ring (formyl label) and a ^{14}C label on the carbonyl carbon of the side chain (acetyl label). Both the nitrofuran part and the hydrazine part of the molecule are of toxicological interest. These studies show that under the approved conditions of use for nihydrazone, residues in edible tissues of chickens, as reflected by measurement of the radioactivity expressed as nihydrazone equivalents, are in the parts per million (ppm) range. Evidence is provided to show that the parent drug, nihydrazone, appears to be only a small fraction of the total radioactivity in the tissue. Other data are presented

to further define the metabolic pathways of nihydrazone.

First, the studies on tissue residues in chickens are discussed. These also include balance studies to account for the percent of administered dose eliminated and that retained in the tissues. Second, the studies relating to the metabolic pathways are discussed. These include studies of urinary metabolites in chickens, rats, and rabbits, as well as synthetic studies, bearing on the questions. The discussion in this second part is organized to present, in turn, evidence for (1) a reductive pathway, (2) a hydrolytic pathway, (3) a pathway involving formation of "415" metabolite, and (4) a discussion of possible ring cleavage.

1. *^{14}C-Nihydrazone studies in chickens.* a. Food and Drug Research Laboratories (FDRL), under contract to Norwich, presented a report dated November 27, 1959, which was submitted in NADA 13–200, Vol. 3. In these studies, white Leghorn cockerels from the hatchery were maintained on niyhdrazone-medicated feed to a weight of 200 grams (20 to 22 days on medication). ^{14}C-nihydrazone (formyl label) was then administered twice daily to three of the cockerels at a daily dose of 4 mg/day. The birds were sacrificed after 2, 4, and 9 days on treatment with the radiolabeled drug.

Norwich stated that the result of the balance study, to account for the percent of administered dose eliminated versus that retained, showed the chick attained body equilibrium with respect to the drug after 2 days and before the fourth day of dosing, and further showed no evidence of residue accumulation in any specific tissue. Food and Drug Administration scientists conclude that with only one bird examined after each feeding period, animal variation in tissue residue levels cannot be determined. Therefore, definitive evidence is not presented to determine if residues are accumulating after 4 days of repeated dosing. However, in the following two examples, it appears that there is an indication of accumulation. The residue levels in the leg muscle for the three birds after 2, 4, and 9 days' dosing, reported as nihydrazone equivalents, were 3.92, 5.79, and 7.01 ppm, respectively. Corresponding values for liver were 9.68, 10.61, and 13.42 ppm. Residues in blood, head, leg muscle, fat, and residual carcass after 9 days on radioactive drug treatment were reported at about 7 ppm and were all greater than the 4-day values. In the reported balance study, recovery of the radioactivity as percent of total dose administered for treatment periods of 2, 4, and 9 days, respectively were: expired carbon dioxide ($^{14}CO_2$) (11.5, 4.3, and 10.9 percent); urine and feces not separated (80.6, 71.5, and 85.0 percent), and tissues (12.1, 6.0, and 5.9 percent). These data will be compared with a later study also utilizing the formyl label (section II.A.1.d. of this notice) and a study utilizing the acetyl label (section II.A.1.b.).

b. Nuclear Science and Engineering Corp. (NSEC), also conducted a study for Norwich, titled "Chicken Residue of ^{14}C-nihydrazone", NSEC No. 50–11–9098, December 4, 1959, submitted in NADA 13–200, Vol. 3. In the NSEC study, 1-day-old white Leghorn chicks were maintained on nihydrazone-medicated feed for 4 weeks before the administration of radiolabeled drug. ^{14}C-nihydrazone (acetyl label) was given twice daily at a dose of 4 mg/day to three birds. The birds were treated with the radiolabeled drug for either 2, 4, or 8 days, and then were withdrawn from medication for 1 day before sacrifice. Recoveries of radioactivity as percent of total dose for the treatment periods of 2, 4, and 8 days, were as follows: expired $^{14}CO_2$ (46.8, 57.4, and 60.9 percent); urine and feces not separated (33.6, 37.9, and 47.6 percent); and tissue (11.78, 7.92, and 10.2 percent). It is apparent from the results on expired $^{14}CO_2$ in the NSEC study that the radioactive carbon derived from the acetyl label is removed from molecular species of toxicological interest and that the radioactivity in the tissue will include incorporation of ^{14}C into endogenous substances that are not of toxicological interest. For this reason, the NSEC study does not answer the questions about the total drug-related residues in tissues of interest and their depletion with time. The results from the formyl label studies discussed in the preceding paragraph also indicate some expired $^{14}CO_2$, but to a lesser extent (4.3 percent to 11.5 percent).

c. Further studies were conducted by J. A. Buzzard et al. and were submitted by Norwich (Special Report Problem No. 360.3, September 1, 1961, NADA 13–200, Vol. 3). In these studies, male and female chicks were administered nihydrazone ad libitum in the feed at 0.0138 percent for 3 weeks. One group was then given ^{14}C-nihydrazone, labeled in the formyl position, and another group was given ^{14}C-nihydrazone labeled in the acetyl position. Radiolabeled drug was given for 4 days; Norwich claimed that previous studies showed that 4 days was the time required for tissue saturation and equilibration. One male and one female chick from each group were sacrified for analysis after radiolabeled drug treatment for 1, 3, or 4 days; there was no withdrawal from treatment before sacrifice.

In the formyl-labeled studies, the maximum levels of radioactive residue after dosing from 1 to 4 days, expressed as nihydrazone equivalents, were approximately 2 to 3 ppm in thigh, breast, and leg muscle; and about 8 ppm in liver and kidney. Fat was only analyzed on day 1 and was 1.35 ppm nihydrazone equivalent. With the acetyl label, the maximum level of radioactive residue after dosing for 1 to 4 days was much higher. For example, maximum values of 6.8 ppm in thigh muscle, 35 ppm in kidney, 15.6 ppm in liver, and 91.3 ppm in fat, expressed as nihydrazone equivalents, were reported. Data were also presented on the depletion of residues after 3 weeks' treatment with 0.0138 percent nihydrazone in feed followed by 4 days of radiolabeled drug treatment and subsequent withdrawal from medication. The formyl-label-treated chicks were analyzed after sequential sacrifices up to 5 days postmedication, and the acetyl-label-treated chicks were analyzed after sequential sacrifices up to 16 days postmedication. One male and one female chick were analyzed from each group at each withdrawal time up to 5 days withdrawal. After 5 days withdrawal, the formyl-label-treated chick had the following residue levels, expressed as nihydrazone equivalents: 0.79 ppm and 0.96 ppm in muscle tissues, 0.70 ppm and 0.86 ppm in fat, 1.27 ppm and 1.64 ppm in kidney, and 1.36 ppm and 1.64 ppm in liver. Liver and kidney showed biphasic disappearance curves indicating a pool of rapidly disappearing metabolites with a biological half-life, calculated by Norwich to be about 0.7 day. The pool of slower-depleting components in the various tissues was reported to have a biological half-life ranging from 3.2 days in liver to 4.9 days in breast muscle. Fat was not examined. Results demonstrating a biphasic nature for the depletion of residues from use of the acetyl label were also presented. A biological half-life for the rapidly depleting pool is reported at about 0.8 to 1.6 days. The biological half-life of the slower-depleting pool was reported as 4.1 days for fat, about 5 days in kidney and liver, and 7.2 to 11.7 days for muscle tissues. Norwich did not explain how it derived the biological half-lives for the rapidly depleting pools.

With the acetyl label, initial residues were much higher than with the formyl label. After 16 days withdrawal, the level of radioactivity in tissues was in the same range as that seen after only 5 days withdrawal with the formyl label.

Further studies were presented in this report to show the effect of longer-term administration of radiolabeled drug on the residues measured. Norwich claimed from the previous studies that dosing with the labeled drug for 4 days was sufficient to assure saturation of tissues with radiolabeled drug residues after pretreatment with unlabeled drug. Three 21-day-old male Leghorn birds, not pretreated with nihydrazone, were given the formyl label drug in feed for 8, 16, or 32 days, after which each bird was withdrawn from treatment for 4 days before sacrifice and measurement of residues. The data combined with the 4-day treatment data from the previous study show that the amount of residue in liver, kidney, fat and thigh, breast, and muscle tissue, expressed as nihydrazone equivalents, continued to increase with increasing length of treament. For example, muscle residues were reported as 0.79, 1.35, 1.78, and 2.17 ppm for the 4-, 8-, 16-, and 32-day treatment, respectively, each followed by a 4-day withdrawal from medication. A similar study with the acetyl label, where drug treatment was given for 8, 16, 24, or 32 days followed by 4 days withdrawal, also indicated an increase in residues with prolonged treatment. However, the pattern seen in the tissues in this study deviates for the kidney and fat tissue in particular. Food and Drug Administration scientists agree in part with Norwich that this deviation probably results from loss of labeled acetate from nihydrazone. This would result in the

incorporation of ^{14}C derived from acetate into endogenous substances. This is consistent with the showing of a much higher $^{14}CO_2$ expiration with the acetyl label compared to the formyl label as previously discussed. It is also consistent with the higher level of radioactivity, especially in fat, for the acetyl versus the formyl-label-treated birds. Acetate is a biochemical precursor of fatty acids, and therefore a higher incorporation of radiolabel into endogenous fatty acids would be expected. Norwich references work by Weinhouse et al. (Journal of Biological Chemistry, 166: 691, 1946) and Elliott et al. (Journal of Biological Chemistry, 186: 477, 1950) to support that kidney in general is a high acetate metabolizing organ, and therefore, as with fat, the high apparent residue levels in kidney may result from the incorporation of radiolabeled acetate into endogenous substances.

Food and Drug Administration scientists agree that kidney is a high acetate metabolizing organ; however, liver also has strong acetate metabolizing capability, and in comparison to kidney, liver has a much more versatile anabolic (synthetic) capability. Therefore, the argument of Norwich that the disproportionate amount of kidney residues results from the labeled acetate is not supported in view of the data for liver and common knowledge of liver metabolism.

d. Norwich has provided an additional report using the formyl label to study residues in chickens. Buzzard et al., in Special Report Problem, No. 370.3, September 1, 1961, reported a radioactivity recovery study in the chicken. One chicken was fed a diet containing nihydrazone at 0.0138 percent for 5 weeks before the administration of ^{14}C-nihydrazone (formyl label) for 8 days. The bird was then withdrawn from medication for 4 days before sacrifice. Recovery of radioactivity as percent of the total dose administered was reported as follows: expired $^{14}CO_2$ (2.52 percent), urine and feces (100.45 percent), and tissues (2.17 percent). Residues in the tissues, expressed as nihydrazone equivalents, ranged from a low of 0.5 ppm in the crop and its contents, to 1 to 2 ppm in muscle tissues, 4.0 ppm is liver, and 4.2 ppm in kidney. The results of this experiment show a lower level of expired $^{14}CO_2$ compared to the results of the FDRL study with the formyl label previously described, where about 11 percent of the total dose was accounted for as $^{14}CO_2$ for two of the three birds examined. In the Food and Drug Research Laboratories study (cited previously in this notice, under section II.A.1.a.), some high values during the early collection period were questioned; investigators felt they might possibly be due to fermentation of droppings during collection.

The report continues with data that Norwich claims demonstrate that the ^{14}C-nihydrazone (formyl label) results in the incorporation of carbon-14 into serum proteins, glucose from liver glycogen, glutathione from erythrocytes, glutamic acid isolated from erythrocyte glutathione, and glutamic acid, serine, tyrosine, aspartic acid, and phenylalanine from blood hydrolysates. These results are based on presumptive identification. After acid hydrolysis of the tissues, ion exchange column chromatography was used to separate the amino acids, and identification was based on column retention time compared to standards. From the evidence of expired $^{14}CO_2$ (although only a small percentage of the total dose), it may be expected that the formyl label also would result in a small amount of incorporation of ^{14}C into endogenous substances. The data presented by Norwich do not provide any estimate of the proportion of the total radioactive residues that result from such incorporation. Therefore, the total drug residues of toxicological concern cannot be determined. As one example, Norwich reported that erythrocytes contained 74 percent of the radioactivity in the blood. The extraction of 1 milliliter of erythrocytes yielded 1.4 milligrams of glutathione with a radioactive count of 10,466 disintegration per minute per milligram (dpm/mg). After hydrolysis and chromatography to separate glycine, cystine, and glutamic acid, it was reported that the radioactivity was associated with glutamic acid representing a radioactive count of 560 dpm, which is only a minor part of the total radioactivity in the isolated glutathione fraction (about 14,600 dpm). Bar graphs were presented for the various amino acid fractions from hydrolyzed blood protein with which radioactivity was associated. The largest component was not identified with the endogenous substances examined.

Food and Drug Administration scientists cannot use such data to determine what amount of radioactive residue has to be subtracted from the total residue in edible tissue to yield that portion that is of remaining toxicological interest. Furthermore, although much of the radioactivity appeared to chromatograph with retention times similar to the amino acid standards, many substances, even including some of the standards, co-chromatograph. Therefore, to presume identification of an endogenous substance solely by column retention time is not considered reliable because it is possible that drug-related metabolites may also chromatograph in the system described. Furthermore, it is possible that some of the nihydrazone metabolite residues may be "bound" in some manner with macromolecular structures and at least in part may be contributing to some of the results seen in the data of this study.

The Director concludes from the studies with the acetyl label that this label does not provide any meaningful answers to the question of drug-related residues of toxicological concern. It appears that the acetyl label may not remain associated with the hydrazine part of the molecule that is of toxicological interest, and would likewise not provide an adequate label for following the fate of the nitrofuran part. The Director concludes that the formyl label more reasonably provides an estimate of the total drug-related residues of interest. Studies, which will be discussed later, indicate that one pathway for nihydrazone metabolism includes breaking the carbon nitrogen double bond in the side chain giving rise to furan derivatives as well as hydrazine derivatives. The latter would not be followed with the formyl label. However, this hydrolytic pathway appears to be significant in the rat and rabbit but is apparently not operant in the chicken, possibly due to different gastrointestinal tract acidity.

The Director further concludes that substantial levels of radioactive residue are shown in the edible tissues of chickens. The Director acknowledges that the formyl label data also indicate that some ^{14}C incorporation into endogenous substances may occur, based on the small amount of expired $^{14}CO_2$. The Director concludes that Norwich's data on this point are presumptive qualitative information without quantitative accountability. Therefore, the Director concludes that even if he agreed that the evidence supports the presence of labeled ^{14}C in the endogenous substances, he is not able to determine what amount of residue may be subtracted from the total residue as demonstrated to be safe; therefore, he cannot estimate the amount of total drug-related residues of interest from the data presented. Because of the evidence suggesting a carcinogenic potential for the drug, even low levels of nihydrazone-related metabolites are significant.

As will be discussed in the second part of section II.A of this notice, there is reason to believe that major portions of the residue are likely to be drug-related metabolites of toxicological concern.

e. Norwich has presented some data on the amount of parent drug residue, i.e., nihydrazone residue, that occurs in chicken tissue. These data are described in detail in section II. F. of this notice. In one study, broiler chickens were treated for 5 days with nihydrazone in their ration at 100 grams/ton. A ^{14}C-nihydrazone (formyl label) was used, and the levels of nihydrazone, parent drug only, were measured by using a reverse isotope dilution technique following the 5-day treatment and for 5 subsequent days of withdrawal. The maximum concentration of nihydrazone at 0-day withdrawal was in skin with adhering fat at a level of 37 parts per billion (ppb), which depleted to a level of 2 ppb after 3 days withdrawal.

Subsequently, other studies in chickens were conducted using chemical assays to measure residues that Norwich claimed were specific for nihydrazone and could detect residues as low as 2 ppb. The method of analysis used was similar to the one subjected to validation trials for furazolidone (NF-180); the method was not shown to be reliable. Data from these studies, which involved longer periods of treatment of the birds with nihydrazone, indicated that the levels of parent drug, nihydrazone, in edible tissues are in the parts-per-billion range. It is therefore apparent that nihydrazone represents only a very small fraction of the total residue in the tissues when com-

pared to the findings from the radiolabeled drug studies.

The Director concludes that Norwich has not provided adequate identification of the residues in edible tissue to provide convincing evidence that the substantial levels of residues reflected by the radioactivity measurements are safe. However, Norwich has provided other data on the metabolism of nitrofurans. From studies of the nature of metabolites in rabbit, rat, and chicken urine and from synthetic studies, Norwich has shown that several pathways may be operant. These data are germane to the Director's conclusion that adequate information has not been provided about the residues in edible tissues of treated animals to conclude that such residues are safe.

2. *Metabolic pathways.* Norwich has provided some data on the chromatographic analysis of urine from chickens dose with ^{14}C-nihydrazone that indicates that several metabolic pathways are involved. They present evidence to show that in the urine, about 5 percent of the administered dose is excreted as nihydrazone, a similar amount as 5-acetamido-2-furaldehyde acetylhydrazone, about 16 to 17 percent as a compound designated as "415" because the ultraviolet (uv) spectra shows an absorption maximum at 415 nanometers and several other unidentified compounds that had been chromatographically separated. These data will be discussed along with other relevant studies in the following paragraphs.

a. *The reductive pathway.* Norwich has provided several references of work by others that show evidence of the reduction of a number of nitrofurans by various body tissues and bacteria. Beckett and Robinson (Journal of Medical and Pharmaceutical Chemistry, 1:155, 1959) postulated that hydrazone derivatives of nitrofurans of the B Type (—CH=N—N)(R)COR) are unstable and that furan ring cleavage occurs upon reduction because they cannot be stablized by hydrogen bonding. These same authors (ibid. p. 135) demonstrated the stability of Type A hydrazone derivatives of nitrofurans(—CH=N—N(H)COR) upon reduction to their amino analogs. They suggest that the NH group alpha to the Schiff's base may be essential for stability of these amino compounds. Beckett and Robinson used bacterial systems, as well as alcohol solutions (highly protonic) for the reductions. Elbetino et al. ("Reduction of Nitrofurans I. Aminofuran," Eaton Laboratories, report submitted in NADA 13-200, Vol. 3) reported on the catalytic reduction of a number of 2-substituted 5-nitrofurans to the corresponding aminofurans, including the reduction of nihydrazone. They avoided the use of exclusively protonic solutions for the reduction. Based on their findings, these authors found that the reduction did not necessarily result in ring cleavage, particularly for the Type B nitrofurans. They found that the 5-nitrofuran compounds could br catalytically reduced and that the amino analogs were easily acetylated. The acetylated amino derivatives were more stable in aqueous solution than the corresponding amino compounds.

They reported the catalytic reduction of nihydrazone to 5-amio-2-furaldehyde acethylhydrazone, which, after reaction with acetic anhydride in the presence of pyridine, produced 5-acetamino-2-furaldehyde acetylhydrazone. This compound had infrared and uv spectra and a paper chromatography retardation factor (R_f) value identical to that of a nihydrazone metabolite found in rabbit urine by Olivard et al. of Eaton Laboratories (discussed below), which was subsequently confirmed as to identity. Elbetino et al. reported that the 5-amiofuran derivative of nihydrazone and other Type A nitrofurans produced through the catalytic reduction were relatively unstable compared to the Type B nitrofurans studies. In contrast to Beckett and Robinson, they postulated a hydrogen-bonded form that might be more susceptible to furan ring cleavage.

Olivard et al. ("Metabolism of 5-nitro-2-furaldehyde acetylhydrazone," NADA 13-200, Vol. 3) reported the presence if 5-acetamido-2-furaldehyde acetylhydrazone and evidence for 5-amino-2-furaldehyde acetylhydrazone in the urine of rabbits that had been given nihydrazone by stomach tube at a dose of 100 mg/kg body weight/day for 5 days.

The 2,4-dinitrophenylhydrazone derivative of 5-acetamido-2-furaldehyde was isolated from the urine of the nihydrazone-treated rabbits after the addition to the urine of 2,4-dinitrophenylhydrazine in hydrochloric acid, and subsequent extraction with ethyl acetate followed by chromatography on alumina. The isolated derivative was identical with an authentic sample by melting point, absorption maxima in neutral and alkaline solutions, infrared-spectra, and R_f values on paper chromatograms. Elemental analysis supported the empirical formula. Additional evidence for the existence of the 5-acetamido-2-furaldehyde acetylhydrazone in urine was shown by the demonstration of ^{14}C in the R_f area corresponding to the authentic sample when ^{14}C nihydrazone (acetyl label) was administered. Thus it was demonstrated that the carbon-nitrogen double bond in the side chain remains intact, at least in part, in the rabbit. Although neither the 5-acetamido nor 5-diacetamido compounds, synthetically produced or isolated from the urine, could be recrystallized to satisfactory purity for elemental analysis, evidence of the identity of the isolated products from the urine is reasonably assured by the spectral data and paper chromatography R_f values. The "diacetyl" derivative was actually a triacetyl compound when including the acetyl group attached to hydrazine. The infrared spectra, however, did not distinguish whether the diacetamido occurs in association with the hydrazine part or the 5-amino part of the molecule. Fresh rabbit urine indicated only traces of nihydrazone and the 5-amino analog; however, the urine of chickens that had been dosed orally with 100 mg/kg body weight/day of nihydrazone showed essentially equal quantities of nihydrazone, 5-acetamido and the 5-amino metabolites.

In a series of studies, Buzzard et al. reported on the chromatographic study of urine from rats dosed with ^{14}C-nihydrazone as well as urine from chickens dosed with ^{14}C-nihydrazone (Problem No. 370.3, September 1, 1960, June 15, 1961, and September 1, 1961, NADA 13-200, Vol. 3).

The studies all show paper chromatographic evidence for the presence of the acetamido nihydrazone derivative in urine. The paper chromatographic profiles of urine are discussed here and will be referred to in succeeding discussions of the different metabolic pathways and species differences and are relevant to the Director's conclusions in section II.B. of this notice.

In this first study, one rat was dosed with 20 milligrams of ^{14}C-nihydrazone (formyl label) and one rat was dosed with 20 milligrams of ^{14}C-nihydrazone (acetyl label). The urine was first analyzed by paper chromatography using *n*-butanol: 95 percent ethanol: 0.5 *N* ammonium hydroxide in a 4:1:1: system (butanol system). Both labels yielded qualitatively similar chromatographs. From one label, presumably the formyl label, the authors found that 14 percent of the urine radioactivity was 5-acetamido-2-furaldehyde acetylhydrazone, 35 to 40 percent was the "415" metabolite that will be discussed later, 6 to 8 percent was an unknown acidic compound (R_f 0.3) and 30 to 40 percent remained at the origin.

Further paper chromatography of the urine samples was carried out in an SDA (specially denatured alcohol) 30: tris buffer system (SDA 30: 0.5M tris-(hydroxymethyl)-aminomethane in a 4:1 ratio). This system provided improved separation of the more polar materials that remained at the origin in the butanol system.

Comparative data for the rats given the acetyl-labeled nihydrazone indicated that only 6 percent of the urine radioactivity was the 5-acetamido-2-furaldehyde acetylhydrazone, 20 percent was the "415" metabolite, and there was no evidence for the unknown acidic compound at R_f 0.3 in the butanol system. Only a trace of nihydrazone was found. After chromatography in the SDA 30: tris buffer system, 14 percent of the activity was attributed to R_f 0.33 compounds, 10 percent to R_f 0.45 compounds, and 21 percent to R_f 0.70 compounds. Two other minor peaks represented about 12 percent of the urine radioactivity. No explanation is given for the apparent differences in results when the formyl label was used. Deficiencies in the use of the acetyl label, which have been previously discussed, would be expected to confound the relative proporitions of residues separated in the chromatographic systems.

Subsequent data were reported using the same chromatographic systems for the urine from a fasted 200-gram chicken given a 28.6-milligram dose of ^{14}C-nihydrazone (formyl label). Fifteen urine samples separated from feces were collected over a 31-hour period. The maxi-

mum excretion of radioactivity occurred between 2 to 5 hours after dosing. After 31 hours, 40 percent of the administered dose had been recovered in either urine or expired $^{14}CO_2$.

Sufficient raw data were not presented to show what percent of total excreted radioactivity may be assigned to each component chromatographically separated. However, from the data presented for two early collections of urine, it appeared that the nihydrazone and the 5-acetamido derivative were each about 4 to 6 percent of the total radioactivity, and the "415" compounds were about 16 to 17 percent of the total radioactivity in the respective samples. About 60 percent of the radioactivity remained at the origin in the butanol system previously described (1.25 to 2-hour collection sample), and about 10 percent of the radioactivity at R_f 0.35 in the butanol system was designated as an unknown acidic compound. By using the SDA 30: tris buffer system, the origin activity in the butanol system was separated into at least three major metabolites with R_f 0.33 (8.7 percent), R_f 0.45 (10.7 percent), and R_f 0.70 (34.2 percent). The 3 to 4.25-hour collection sample was analyzed for these data. The R_f 0.70 zone in this system also contained the unknown acidic compound with R_f 0.35 in the butanol system. The unidentified compound at R_f 0.35 in the butanol system was not acetylhydrazine, 5-nitrofuric acid, nor the endogenous substance, α-ketoglutaric acid.

The Director concludes that these data conclusively demonstrate a reductive pathway in chickens, rats, and rabbits as shown by the presence of the acetamido derivative of nihydrazone. Hence, the reduced compounds are not necessarily subject to hydrolysis of the carbon nitrogen double bond of the side chain or to ring cleavage, as will be discussed later. The reduced compounds are shown to be partially eliminated as the reduced intact drug. The Director concludes that the potential presence of reduced nihydrazone analogs and derivatives in edible chicken tissues may well be of major toxicological significance because there is reason to believe such compounds may be biologically active when ingested.

b. *Hydrolytic pathway.* A review of the literature on hydrazine derivatives indicates two usual pathways of metabolism in mammalian species. One is *N*-acetylation and the other is a hydrolytic pathway breaking the carbon-nitrogen double bond of hydrazones. By the hydrolytic pathway, nihydrazone would be metabolized to 5-nitro-2-furaldehyde and acetylhydrazide. These pathways influence toxicity. The *N*-acetylation appears to be a detoxification pathway. Monacetyl hydrazide appears consistently to have toxic properties that may approach those of hydrazine. However, 1,2-diacetylhydrazide produces fewer or diminished toxic reactions. Conversely, for the hydrazone derivatives, the toxicity is generally greater in those species where a hydrolytic cleavage of the carbon-nitrogen double bond occurs.

Free hydrazine and various hydrazine derivatives have been reported as carcinogenic in mice, rats, or hamsters (*Biancifiori Tumori* 60:429, 1974 and *Cancer Research*, 35:3693, 1975). The monoacetyl and diacetyl metabolites of hydrazine have not been tested for their carcinogenic potential.

Norwich presented work by McKennis et al. (Journal of Pharmacology and Experimental Therapeutics, 126:109–116, 1959) that included a discussion of previous work by others on the metabolism of certain hydrazines. McKennis et al. have shown that hydrazine and monoacetyl hydrazide on interperitoneal injection to rabbits are both metabolized to 1,2 diacetylhydrazide.

Olivard et al., in a previously referenced work on the metabolism of nihydrazone in the rabbit, reported that following oral administration of nihydrazone, the urine collected contained 5-nitrofuroic acid based on the paper chromatographic R_f value and spectral data, and also contained 1,2-diacetylhydrazide. The 1,2-diacetylhydrazide was identified by R_f value and Ehrlich reagent with subsequent column purification, crystallization, and demonstration of the identity by infrared spectra, R_f value, melting point, and mixed melting point (no depression) with authentic standard. The authors stated that no 1,2-diacetylhydrazide, acetylhydrazide, or 5-nitrofuroic acid was found in urine by paper chromatography if the rabbits were given nihydrazone interperitoneally. Similarly, they found no evidence by paper chromatography of 1,2-diacetylhydrazide, acetylhydrazide, or 5-nitrofuroic acid in the urine from a chicken given nihydrazone orally. The authors suggest that the hydrolysis of the carbon-nitrogen-double-bond linkage of the side chain is related to the liability of nihydrazone under conditions of acidity comparable to those in the mammalian stomach, and that it is unlikely to occur under the weakly acidic conditions of the gastrointestinal tract of the chicken.

It was shown by studies with chickens using the ^{14}C-nihydrazone (acetyl label) discussed in the first part of section II.A. of this notice, that a large amount of $^{14}CO_2$ was recovered in expired air as opposed to a much smaller amount of $^{14}CO_2$ recovered when the formyl label was used. Therefore, it would appear from this evidence and considering the evidence presented in the above studies by Olivard et al. that another type of hydrolysis may occur in the chicken. It appears that in the chicken, deacetylation might be a major hydrolytic pathway that was not discussed in the data presented by Norwich. Some compounds are known to be *N*-deacetylated in poultry, as evidenced by reports by S. A. Peoples and G. L. Westberg, Federation Proceeding, 34:227, 1975, and R. H. Nimmo-Smith, Biochemical Journal, 75: 284, 1960. The formation of deacetylated nihydrazone or its corresponding reduced analogs or derivatives in the chicken may result in tissue residues. Such residues might then be subject to hydroylsis in the gastrointestinal tract of man, involving breaking the carbon-nitrogen double bond, giving rise to hydrazine as well as potentially toxic nitrofuran metabolites.

The data presented by Norwich on nihydrazone show that a hydrolysis, breaking the carbon-nitrogen double bond of the side chain, is operant in the mammalian systems but does not appear to be operant in the chicken.

The Director concludes that hydrolytic pathways of two types may occur. The first type, deacetylation, has been indicated in the chicken by the large amount of expired $^{14}CO_2$ when the ^{14}C-nihydrazone (acetyl label) was orally administered compared to use of the formyl label. Although N-acetylation is generally the usual pathway for hydrazine metabolism is mammalian species, there is evidence for deacetylation of some drugs in poultry. The second type of hydrolysis, involving breaking the carbon-nitrogen double bond, has been shown in the rabbit. However, this hydrolysis does not appear to be operant in the chicken; this observation is based on the lack of evidence for 5-nitrofuroic acid, and mono- or diacetyl hydrazide as urinary metabolites. The Director concludes that deacetylated nihydrazone, and its reduced analogs or derivatives are of concern as potential tissue residues in chickens. Studies have not been presented by the sponsors of nihydrazone to show that the substantial levels of radioactive residues in chicken tissues are not due to the presence of such compounds. The Director further concludes that deacetylated nihydrazone deriyates, if present in chicken tissues, upon ingestion by man would likely be subject to hydrolysis, breaking the carbon-nitrogen double bond, as indicated by the evidence for this pathway in mammalian species. This probability presents serious questions about man's potential exposure to free hydrazine, which is a demonstrated carcinogen, in addition to exposure to other potentially toxic derivatives of the 5-nitrofuran part of nihydrazone.

c. *Pathway involving formation of "415" metabolites.* A metabolite yielding a uv absorption maximum of 415 nanometers was found in rat, rabbit, dog, monkey, and chicken urine after dosing with nihydrazone according to the report by Olivard et al., previously referenced. The "415" metabolite did not appear to result from either the simple hydrolysis of the carbon-nitrogen double bond or from the reductive pathway, yet both a nitro group and a carbon-nitrogen double bond are apparently necessary for the formation of the "415" metabolite. Administration of "415" metabolite to the rat did not yield reduced nitrofurans nor did dosing with the reduced nitrofurans yield "415." The infrared spectra of "415" metabolite indicated a carboxyl anion, two carbonyl groups, a carbon double bond with no furan, nitro or aromatic group. The spectroscopist indicated there was a probability for the presence of OH and NH, but not much CH.

The compound with mild acid treatment instantly was converted to one with

an R_f of 0.5 in the butanol paper chromatographic system previously described and had an absorption maximum of 400 nanometers. It is in turn gradually destroyed by 2.3N hydrochloric acid at 70° C. Chromatographs of the hydrolyzate showed no ninhydrin positive zones, as would be expected for amino acids. Preparation of the 2,4-dinitrophenylhydrazine derivative of "415," as indicated by the infrared spectra, gave a compound showing only one absorption peak in the carbonyl region in contrast to the 2 carbonyl peaks seen in the infrared spectrum of "415." The "415" metabolite can apparently be made photochemically from 5-nitro-2-furaldehyde diacetate and coupling with acetone acetylhydrazone. It is believed that the "415" compound retains the carbon-nitrogen double bond of the side chain and thus would include the hydrazine part of the molecule. Buzzerd and Olivard proposed that the "415" compound may be related to the Stenhouse dyes formed from aniline and furfural. However, all attempts by the sponsors to identify the "415" metabolite, which appears to be the major residue component in the urine of animals dosed with nihydrazone, have been unsuccessful.

The Director concludes that a pathway involving formation of "415" metabolite is operant in chickens and mammalian species, and the evidence indicates this metabolite is drug related and not an endogenous substance. Its potential presence as a tissue residue and a part of the total radioactive residue seen in chicken tissue has not been resolved.

d. *Furan ring cleavage.* Buzzard, Hoetis and Klein (Problem No. 370.3, October 1, 1961, NADA 13–200, Vol. 3) showed that by alkali-catalyzed hydrolysis of ethyl-5-amino-2-furoate, followed by acid hydrolysis of the products of the ring opening reaction, α-ketoglutaric acid was formed, identified as the 2,4-dinitrophenyl hydrazone derivative) as well as β-formyl propionic acid. A mechanism for the reaction was proposed. Since attempts to prepare 5-amino-2-furoic acid were not successful, it could not be shown whether nihydrazone would react similarly. Based on the observed chemical activity of the model furan studied, Norwich hypothesized that the slower depleting pool of residues in chickens dosed with nihydrazone arose from a similar degradation of nihydrazone through formation of α-ketoglutaric acid. The work of Buzzard et al., discussed in section II.A.1.d. of this notice, which presents presumptive evidence for the incorporation of ^{14}C into endogenous substances in chicken tissues, in the opinion of Norwich, adds further support to the hypothesis.

There is no evidence to show that the chemical reaction of the model furan giving rise to α-ketoglutaric acid is a reaction that would occur in the chicken. Investigation of the "415" metabolite in urine also gave some evidence that ring cleavage may be involved. However, as previously discussed, "415" is not an endogenous substance.

Even if α-ketoglutaric acid were one of the products of nihydrazone metabolism, there is question about what part of the radioactive residues in chicken tissues would be attributable to this hypothetical pathway. As discussed in section II.A.1.d. of this notice, the evidence provided by Buzzard et al. for the identification of the radioactive residues in chicken tissues as endogenous substances is presumptive and does not provide quantitative accountability.

Therefore, the Director does not accept the evidence provided by Norwich as sufficient to show that the residues persisting in chicken tissues after a short withdrawal period are the result of nihydrazone metabolism to endogenous substances through ring cleavage and formation of α-ketoglutaric acid. Norwich has provided evidence in animals that ring cleavage of nihydrazone may occur in the formation of "415" metabolite that is not an endogenous substance. Norwich has not provided conclusive evidence that ring cleavage of nihydrazone occurs in chickens to produce the formation of α-ketoglutaric acid. Even if this pathway did occur, no quantitative accountability of residues identified as endogenous substances has been provided. Furthermore, a pathway by which nihydrazone would be degraded to α-ketogolutaric acid would require hydrolysis of the carbon-nitrogen double bond. Therefore, the fate of the hydrazine part of the molecule would also have to be accounted for. Studies provided by Norwich indicate that this hydrolysis is not prevalent in the chicken. Therefore, the Director concludes that a pathway involving furan ring cleavage does not necessarily lead to metabolities that may be presumed safe without supporting evidence.

In summary, the Director concludes that each of the studies presented in this part adds to knowledge of the metabolism of nihydrazone, but the studies do not satisfactorily answer the significant questions about the safety of the residues. There is a demonstration of a reductive pathway leading to the formation of compounds of toxicological concern. There is also evidence of a possible deacetylation pathway in the chicken that leads to the potential for compounds which, as residues, if ingested by mammalian species may result in exposure to free hydrazine, which is a carcinogen, and other potentially toxic nitrofuran analogs and derivatives. There is further demonstration of a major pathway involving formation of "415" metabolite; it appears to result from ring cleavage but with the hydrazine part of nihydrazone intact. From all of these factors and the lack of information on tissue residues arising from these proposed pathways of metabolism, the Director concludes that the substantial levels of radioactive residues in chicken tissue even after a 4- or 5-day withdrawal period are not shown to be safe. There is no conclusive evidence that nihydrazone is degraded to α-ketoglutaric acid in the chicken. Nor is there concurrent accountabiilty for the hydrazine part of nihydrazone in this proposed pathway. Even if the identification of some radioactive residues in chickens as endogenous substances, based on column chromatography, were accepted, quantitative accountability for these residues is lacking. Therefore, the Norwich contention that nihydrazone residues in chicken tissue, following the currently labeled conditions of use, are endogenous substances and therefore safe, cannot be accepted by the Director.

B. *Sufficient Information Has Not Been Provided about the Residues to Determine the Appropriate Testing Required in Laboratory Animals to Estimate the Cumulative Effects of Human Exposure to the Residues (Step 2).* Norwich has demonstrated that very little of the residue in edible tissues is nihydrazone and most of the residues are metabolites.

The Food and Drug Administration must assess the toxicity of the total residue because this is what man will be exposed to in food. This assessment would be based ideally on the identification of each of the metabolites in the residue, their synthesis, and subsequent testing of the parent drug and all the metabolites for the cumulative effects of exposure. However, this approach is usually impractical.

Recognizing this, FDA makes judgmental assessments from certain types of data about the residue and metabolism. A determination is made about assumptions that can be accepted from the toxicity testing of the parent drug. Because the test animal metabolizes the drug, the resulting dose-response effects represent to some degree the cumulative effects of exposure to each of the biologically active substances produced by the test animal.

Norwich has provided some comparative metabolic data in the rat and chicken through chromatographic analysis of the urine collected after treatment with labeled drug. These data show certain qualitative similarities between the two species. Both the chicken and rat appear to excrete "415" metabolites and 5-acetamido-2-furaldehyde acetylhydrazone; this indicates that both the reductive pathway and the pathway leading to formation of the "415" metabolite are involved in both species. The proportion of the total radioactivity in urine for each of the compounds appears to be somewhat higher for the rat than for the chicken. Only trace quantities of nihydrazone are reported in rat urine, whereas nihydrazone was about 5 percent of the total radioactivity in early collection samples of chicken urine, roughly equal to the amount of 5-acetamido-2-furaldehyde acetylhydrazone excreted in urine. It was also reported that an acidic compound with an R_f at about 0.3 in the butanol chromatographic system represented about 10 percent of the radioactivity in chicken urine. This compound was also reported in the urine of the rat treated with the formyl label, but the compound was not seen with the acetyl label. Substantial quantities of unknown compounds were seen in both rat and chicken urine which remained at the

origin in the butanol system and were further separated in the SDA 30: tris buffer system, indicating that these materials represented more polar metabolites.

One major difference is apparent in the chicken versus mammalian species. This involves the hydrolytic pathways previously discussed. Deacetylation is an apparent major pathway in the chicken, and there is no evidence for a hydrolytic pathway in the chicken that involves breaking the carbon-nitrogen double bond. Conversely, there is evidence that a hydrolytic pathway with breaking of the carbon-nitrogen double bond is operant in the mammalian species, and that the resulting hydrazide is acetylated. The scientific literature suggests that deacetylation in mammalian species is not a significant pathway.

Although the sponsors have not provided information characterizing the tissue residues, it may be assumed that at least some of the metabolites found in chicken urine may also be present as tissue residues. The "415" metabolite and at least some of the reduced nitrofurans appear as urinary metabolites in the chicken as well as in mammalian species. Therefore, in testing nihydrazone in the rat, some exposure of the rat to these metabolites would have occurred. However, chicken tissue may contain deacetylated metabolites of nihydrazone. Testing of nihydrazone in the rat is unlikely to have provided exposure to deacetylated nihydrazone or deacetylated derivatives of nihydrazone formed through other pathways operant in the chicken. Both the nitrofuran part of nihydrazone as well as the hydrazide part of nihydrazone may be involved with tumorigenesis. In general, acetylation appears to be a detoxification pathway for hydrazines. Therefore, there is some reason to believe that deacetylated nihydrazone metabolites may be more toxic than nihydrazone when ingested by mammalian species.

In order to make an adequate assessment of the extent to which testing of nihydrazone can be used to evaluate the safety of residues, sufficient information about the residues in chickens is required in addition to comparative metabolic data in the test species. Because the nature of the residues in chicken tissues is not known, there are unanswered questions about potential residues, including those residues that may give rise to exposure to free hydrazine when ingested. Therefore, the Director concludes that not only is the toxicological testing provided for nihydrazone inadequate, but he is unable to determine whether testing of metabolites in addition to the parent drug is required to determine the safe level of human exposure to residues in food. Step 2 has not been fulfilled; therefore, the requirements for step 3 cannot be fully determined.

C. *Adequate Dose-Response Data in Test Animals to Determine the Cumulative Effects of Dietary Exposure to the Residue Compounds Has Not Been Provided; Therefore, the Maximum Acceptable Concentration of Residues in Food Cannot be Established Using Appropriate Safety Factors to Assure That Human Exposure to Residues in the Total Diet Does Not Exceed a Level Deemed Safe (Step 3).* As discussed under section I of this notice, the Director has concluded that adequate testing of the parent drug, nihydrazone, has not been provided. Also, under steps 1 and 2, discussed in section II. A. and B. of this section, the Director concluded that adequate information has not been provided about the residues to determine if toxicity testing of residue compounds in addition to the parent drug is required to estimate conservatively the cumulative effects of exposure to the residues.

The Director concludes that without such essential information about the residues and adequate toxicity testing data, he cannot, in light of the new evidence, determine if nihydrazone requires regulation as a carcinogenic animal drug or, alternatively, to reasonably estimate a maximum acceptable level of residue in food that can be deemed safe. Therefore, he concludes that adequate information has not been provided to assess the cumulative effects of exposure and to determine the appropriate safety factors required under section 512(d)(2)(B) and (C) of the act.

D. *Sufficient Information about the Residues Has Not Been Provided to Establish the Conditions Necessary for the Analytical Method Used to Control Residues (Step 4).* The evaluation of an analytical method for residues first requires a determination of the tissues to be analyzed, the compound to be measured, and the lower limit of mesurement that must be achieved to control total residues adequately. Only then, after selection of these parameters, is it meaningful to consider whether the method proposed by the sponsor meets other characteristics necessary for a practicable reliable method.

1. Determination of the target issue: The most practical approach to monitoring and controlling residues in the field is dependent on the selection of that edible tissue in which residues require the longest time to deplete to a "safe concentration." Therefore, by monitoring this tissue to assure that residues are below a "safe concentration," it can be assured that all residues in the carcass are below "safe concentrations." In lieu of this determination (identification of the target tissue), all edible tissues, including milk where applicable, must be monitored. This would place an additional burden on public funds for monitoring.

The target tissue is readily identified if the requirements of step 1 of the evaluation process are met. Although Norwich has presented data on the total residues, as measured by radioactivity and expressed as nihydrazone equivalents, these data show substantial amounts of residues and do not show depletion to levels that might be presumed safe, considering that nihydrazone is a tumorigen and may be a carcinogen. From the radiotracer studies it would appear that liver or kidney may be the target tissue. On the other hand, data on the measurement of parent drug, nihydrazone, indicate that skin with adhering fat shows residues at the highest level for the longest time for this residue component. Because adequate data have not been provided to determine when total drug residues of toxicological interest are reduced to levels that can be presumed safe, and depletion characteristics of the component residues are not provided relative to the total residues down to an established safe level, it is not possible to make an assignment of the target tissue.

2. Determination of the compound (marker) that can be measured by the analytical method to assure control of total residues: Because the radiolabeled drug approach used initially to estimate total residues is not applicable to regulatory use, an alternative approach must be developed to assure control of total residues after drug approval. This is achieved by selecting an appropriate residue compound, which may be the parent drug or one or more metabolites, in such a way that its measurement serves as a valid indicator that when the marker compound is below a certain level in the target tissue, all residues in edible tissues are at safe levels. This step is essential to determine if the method of analysis for regulatory use is adequate. To determine a valid marker, adequate information, e.g., information derived from conducting an adequate metabolism study, is required to relate the proportionate amount of each of the residues to the total residue and their change in quantitative relationships with time. The marker must be a known proportion of the total residue when the total residue depletes to the "safe concentration" in the target tissue. It also must be structurally identified so that an adequate and specific method of analysis can be developed for its determination.

The regulatory method proposed by Norwich to control residues uses the parent drug, nihydrazone, as a marker in each of the major edible tissues. Norwich has failed to submit substantiating evidence to show that the measurement of nihydrazone is adequate to control total residues of potential concern or, alternatively, to show that all other residues are safe and do not need to be controlled.

3. Determination of the lower limit of measurement required for the analytical method: Establishment of the lower limit of measurement required for the assay begins with the availability of adequate toxicological testing to determine the cumulative effects of exposure. As described in section I of this notice, the sponsors have not provided adequate toxicological testing of nihydrazone. Also, as described in steps 1 and 2 in section II.A. and B. of this notice, the sponsors have not provided adequate information about the substantial levels of residue shown by radiotracer studies to show if portions of the residue can be removed from toxicological concern, and to adequately define the residues of interest to determine if testing of any of

the metabolites in addition to the parent drug, nihydrazone, is required for a determination of safety. Therefore, lacking this essential information, the Director concludes that he is unable to make an appropriate estimate of the acceptable level of exposure to total residues of interest in food.

Even if adequate testing were available to estimate the acceptable level of exposure to total residues in food, Norwich has not provided adequate information about a marker compound to determine the lower limit of measurement required for the assay.

As previously stated, Norwich has proposed that the measurement of nihydrazone is adequate to assure control of residues. The proportional relationship of the amount of nihydrazone to the amount of total residues indicates that nihydrazone is even less than 1 percent of the total residue during the early periods of withdrawal. It is therefore unlikely that nihydrazone can be used as a suitable marker unless it were shown that the major portion of the residue, reflected by the radioactivity measurements, can be qualitatively and quantitatively defined to show that these residues are of no toxicological interest. And if the residues cannot be shown to be of no toxicological interest, the lower limit of measurement that would have to be placed on nihydrazone to assure no significant residues from the use of this tumorigenic and potentially carcinogenic drug would probably be beyond technological achievement.

When a "safe concentration is assigned for the total residue of interest, and a suitable marker is selected and its proportional relationship to the total residue at the "safe concentration" is known, one further adjustment is necessary to determine the lower limit of measurement required for the assay. This last adjustment takes into account the degree to which the method of analysis recovers the residue from the sample. Minimum standards for recovery for the acceptance of the method take into account that 100 percent recovery is rarely achieved and that a combined evaluation of the precision and accuracy for measurement of the marker must be considered.

Norwich is aware of the principles just described for the selection of a target tissue, marker compound, and the adjustments required to establish conditions for the assay; this is evidence by Norwich's citation of a paper prepared by two FDA scientists in a submission for furazolidone (NF–180) to FDA on November 11, 1975. The cited paper by M. K. Perez and N. E. Weber, entitled "Requirements for Metabolic Studies—Impact on Food Safety Regulation" was presented at the Gordon Research Conference on Drug Metabolism, June 23–27, 1975.

The Director concludes that he cannot make an assessment of the toxicity, including the carcinogenic potential and potency, of nihydrazone without adequate testing data; he cannot conclude that nihydrazone serves as a valid marker for control of total residues of toxicological interest; and therefore he cannot without adequate supporting data determine the conditions necessary for the analytical method used to control residues. The requirements of step 4 have not been fulfilled. He concludes that the new evidence on nihydrazone (F–64) and furazolidone (NF–180) leads to a determination that the methods of analysis for residues of nihydrazone (NF–64) are not shown to be adequate.

E. *Practicable Methods of Analysis are Not Available to Assure that Total Residues in the Daily Diet of Humans Do Not Exceed a Level Deemed Safe (Step 5).* Section 514.1(b)(7) (21 CFR 514.1(b)(7)) requires the sponsor of a new animal drug application to provide a practicable method of analysis to be used for the control of residues of its product. A "practicable" method is defined by FDA as one that reliably measures the compound throughout all residue levels of interest, and also is sufficiently rapid and reasonable in terms of the expertise and equipment required to be applicable for use in the surveillance and control of residues. "Reliability" is a combination of accuracy, precision, and specificity. Accuracy and precision are quantitative terms. "Accuracy" is the measure of deviation from the true value, and "precision refers to the variability of measurement in replications either by one analyst or within a laboratory or between laboratories. A method may provide reliable measurement if the measurements are not too variable even though it measures less than or more than 100 percent of the true value. Therefore, accuracy and precision must be considered together.

"Specificity is a qualitative term. The measurements obtained by the method must reflect only the compound of interest. Because the method will be used on samples from animals for which exposure history is unknown, the techniques used in the method must be adequate to assure not only the integrity of the measurements, but also the identity of the residue to the exclusion of other itnerfering substances. Therefore, when the quantitative measurements are made by techniques that are not inherently specific, a confirmatory test is required as part of the official method to assure the identity of the residue. The most recently submitted methodology for nihydrazone is based on chromatographic separation and fluorometric determination, which are both nonspecific techniques.

The uses of nihydrazone were approved prior to that time when regulatory methods of analysis for residues were required. In 1962, a method of analysis for tissues capable of measuring nihydrazone as low as 0.1 ppm was submitted in Food Additive Petition No. 665. The method is based on acid hydrolysis to break the carbon-nitrogen double bond, generating 5-nitro-2-furaldehyde and reaction with phenylhydrazone to form the 5-nitro-2-furfural phenylhydrazone derivative, which is measured colorimetrically.

This method was not subjected to validation trials by FDA because it predated this current FDA preclearance requirement. The data submitted in support of this method only included studies of the recovery of nihydrazone from samples to which known amounts of the drug had been added (spiked samples). Recoveries of about 50 percent were estimated by FDA for samples fortified at 0.1 ppm. No evidence has been submitted to show that the method will satisfactorily recover nihydrazone when the residue is naturally incurred through drug treatment (dosed samples) at a level of 0.1 ppm. Furthermore, the method is not specific.

After FDA received reports that nitrofurans had induced tumors in female rats, the drug sponsors were advised in 1969 and officially informed in a conference held January 4, 1971, that the residue methods were no longer deemed adequate. They were told that methods capable of measuring at least 2 ppb of the drug in edible tissues were required. In response to the notices of opportunity for hearing, Norwich submitted newly developed methods of analysis.

For the measurement of tissue residues, Norwich concentrated primarily on the method for furazolidone (NF–180) in the belief that this method could be readily adapted for the measurement of tissue residues for the other nitrofurans.

A discussion of the results on the evaluation of the furazolidone (NF–180) method for tissue analysis (including swine liver, kidney, muscle, and fat and poultry liver, kidney, muscle, and skin with adhering fat) is presented in the May 13, 1976 FEDERAL REGISTER notice for furazolidone. In that notice, the Director concluded that the methods for edible tissues were not shown to be adequate.

Although the drug sponsors have submitted far more information to support the adequacy of the methods of analysis for furazolidone (NF–180) than they have submitted on methods of analysis for nihydrazone, the sponsors have in the intervening years submitted some progress reports on the development of analytical methods for nihydrazone.

In 1971, three different progress reports were submitted on the analysis of nihydrazone in chicken tissues:

1. Project No. 476.17 Interim Report, "Chemical Method for the Determination of Concentrations of Nihydrazone in Chicken Liver at 2 ppb," submitted to FDA by cover letter dated June 30, 1971.

The method presented was based on a fluorometric-densitometry measurement similar to the method submitted for furazolidone (NF–180); the method was not shown to be reliable in the validation trial in 1973. Farrand integrator counts were provided for five control chicken liver samples and for chicken liver samples fortified with 2 ppb of nihydrazone. Norwich reported an approximate recovery of 40 percent using an external standard curve and making corrections for aliquot losses. Food and Drug Administration chemists estimated the actual recovery from these data to be about 28 percent. Individual recoveries for each

sample were not provided by Norwich. The sponsor was notified that these data were not acceptable.

2. By letter dated May 17, 1971, Norwich provided data on the recovery of nihydrazone from chicken liver, kidney, and mucle samples. The same fluorometric-densitometry method was used. Norwich reported the following recoveries and standard deviations from tissues fortified with 2 ppb of nihydrazone: liver (78±31 percent), with a range of 35 to 102 percent; kidney (85±26 percent), with range of 43 to 121 percent; and muscle (77±18 percent), with a range of 30 to 96 percent. These recoveries were corrected for aliquot losses and losses in partitioning between solvents. Therefore, they appear higher than the actual recoveries. The Food and Drug Administration has concluded that the results are too erratic and too low for an acceptable method of analysis.

3. On June 30, 1971, in a letter to the FDA hearing clerk, Norwich submitted an interim report on Project No. 476.17, June 29, 1971, "Chemical Method for the Determination of Nihydrazone in Chicken Skin with Fat at 2 ppb." This project used the same fluorometric-densitometry method used for the other two submissions. Data were presented for 7 control samples of skin with adhering fat and for 21 samples fortified with 2, 4, or 6 ppb of nihydrazone. No recovery data were provided and none could be calculated in the absence of a standard curve. The integrator counts were variable, and the result for one of the 4-ppb-fortified samples was below several of those reported for the 2-ppb-fortified samples. A similar overlap of values for the 4- and 6-ppb-fortified samples was observed. These data were rejected as inadequate. No further data have been supplied by the drug sponsors on methods of analysis for nihydrazone.

None of the submitted methods of analysis for nihydrazone, i.e., the colorimetric or fluorometric-densitometry methods, are inherently specific. No confirmatory tests have been provided by the sponsors that can be used to confirm the identity of any residue found by the proposed assay methods.

The Director concludes that the sponsors have not shown that adequate practicable methods of analysis for residues of nihydrazone in edible chicken tissue are available. Appropriate data have not been provided to determine if nihydrazone can be used as a marker to control total residues in edible products, nor to determine the lower limit of measurement required for a method to assure that total residues do not exceed safe levels. The Director concludes that the methods proposed by the sponsors for the determination of nihydrazone are not quantitative and are not adequately specific at the levels of measurement that are likely to be necessary, based on the tumorigenic property of the drug. Therefore, the methods proposed are not quantitatively or qualitatively reliable and practicable methods for regulatory use, even if it were established that measurement of nihydrazone at 2 ppb was adequate to control total residues of toxicological interest. Therefore, step 5 and the requirements of section 512(b)(7) of the act have not been met.

F. *Practical Conditions of Use Cannot be Prescribed on the Label of Products to Assure Safe Use (Step 6).* Section 512(b)(8) of the act requires that conditions of use assure there are no unsafe residues, and section 512(d)(2)(D) of the act adds a further requirement that these conditions be such that they are "reasonably certain to be followed in practice."

It is required by FDA that residue depletion studies be conducted for each drug use under simulated field use conditions to develop data for establishing the withdrawal period. A withdrawal period is the time following drug treatment when, because of residues, a treated animal cannot be slaughtered for food. A withdrawal period must be sufficiently long to assure adequate residue depletion, but it cannot be so long that it is impractical and unlikely to be followed in practice. An adequate analytical method for residues, preferably the same method that will be approved for regulatory use, must be used to measure the residues in the depletion studies.

Because Norwich has not provided adequate data to determine the requirements necessary for an analytical method to control total residues of toxicological concern, depletion studies under those conditions currently required by FDA for the establishment of an adequate withdrawal period cannot be designed. Norwich, through its radiotracer studies, has shown that substantial levels of residue, expressed as nihydrazone equivalents, are present in edible tissues following drug treatment and persisting through the longest periods of withdrawal (5 days with the formyl-labeled nihydrazone). Although radiolabeled studies may be used initially to estimate drug depletion, it is usually not practical to follow this approach for the final determination of the withdrawal period. The establishment of a withdrawal period requires consideration of animal-to-animal variability, thus requiring that larger numbers of animals be used for the determination of residues. Likewise, it is important to have data using the chemcal method proposed for control of residues and to compare these results with the results from the radiotracer metabolism studies to evaluate the capability of the method satisfactorily to recover the residue when naturally incurred.

Norwich has failed to show through the radiotracer studies that a safe and practical withdrawal period can be established for the uses of nihydrazone. Radioactive residues, expressed as nihydrazone equivalents, are shown in the few animals examined to be at levels of 1 to 13 ppm in tissues after treatment with the ^{14}C-formyl-labeled nihydrazone. They are shown to persist in muscle tissue at levels just below 1 ppm and in other major edible tissues at levels from 1 to 2 ppm following treatment of birds on the approved level of drug for 3 weeks and subsequent dosing with the radiolabeled drug for 4 days and observation of a 5-day withdrawal period.

Norwich has provided data in 3 reports on the depletion of parent drug, nihydrazone, from edible tissues:

1. Problem No. 476.03, Final Report "NF-64 and Metabolite Residues," September 24, 1969, NADA 13-200V, Vol. 5. ^{14}C-Nihydrazone (formyl label) was given to broiler chickens in their ration at 100 grams per ton for 5 days. The birds were then maintained on unmedicated feed, and four birds were sacrificed each day thereafter, up to 5 days after withdrawal from medication. A reverse isotope dilution technique was used to determine parent drug, nihydrazone, in muscle, liver, kidney, and skin-with-fat tissues. Norwich claimed the reverse isotope procedure was capable of measuring nihydrazone as low as 2 ppb. At 0-day withdrawal the levels of nihydrazone were reported as follows: muscle (less than 2 ppb to 9 ppb), kidney (4 ppb to 23 ppb), skin with fat (3 ppb to 37 ppb), and liver (less than 2 ppb and 3 ppb). Norwich reported that no nihydrazone was detected at the other withdrawal times, except for skin with fat. One skin-with-fat sample out of four examined after 3 days withdrawal indicated 2 ppb of nihydrazone.

2. Research Report No. JMS 63:1, "Nihydrazone Residue Analysis of Tissues of Chickens Receiving Rations Containing 100 g Nihydrazone per Ton of Feed for Eight Months," August 27, 1963, NADA 13-200, Vol. 5, from letter of submission to FDA January 8, 1971. Edible tissues from five birds given nihydrazone in the feed at 100 grams per ton for 8 months were analyzed using a colorimetric procedure. Norwich claimed the colorimetric procedure could detect nihydrazone at levels as low as 0.1 ppm. Norwich reported that all tissues contained less than 0.1 ppm of nihydrazone at 0-day withdrawal. Positive values for one liver and one kidney sample were reported but were dismissed by Norwich as false positives because of the lack of the characteristic pink or red color generally observed for the 5-nitro-2-furfural phenylhydrazone derivative during column chromatography.

3. "Tissue Residue Withdrawal Studies for Nihydrazone (Sonifur) in Chickens Fed Nihydrazone at 100 Grams Per Ton of Feed for Eight Weeks," from a Memorandum from Dr. Mertz to Dr. Heotis, November 9, 1971, submitted to FDA by cover letter of November 10, 1971, NADA 13-200V, Vol. 5.

Edible tissues from chickens fed a ration containing nihydrazone at 100 grams per ton for 8 weeks were analyzed by the fluorometric-densitometry method described in section II.E. of this notice, Norwich claimed the fluorometric-densitometry method could measure nihydrazone as low as 2 ppb. Samples of liver, muscle, kidney, and skin with fat from birds sacrificed at 4 and 5 days after withdrawal from medication were examined. One out of eight liver samples after 5 days withdrawal was reported to contain nihydrazone at 2 ppb. One out of seven skin-with-fat samples examined after 5 days withdrawal from medication was reported to

contain nihydrazone above 2 ppb, and an additional sample was reported questionable.

The Director concludes that withdrawal periods are necessary for the safe use of nihydrazone. The currently labeled withdrawal period for chickens now voluntarily used by the sponsors is 4 days. The Director concludes that data to support this withdrawal period or any practical withdrawal period has not been provided because of the following reasons:

a. The data show that the levels of total residue, from the limited radiotracer studies available, are substantial and not shown to be safe.

b. The data on depletion of nihydrazone where larger numbers of birds were used are not adequate because no data have been provided to show whether the measurement of nihydrazone in edible products can be used to control total residues of toxicological concern and, if so, at what level its measurement is required to assure that all residues are below concentrations deemed safe.

c. The chemical methods used to determine the depletion of nihydrazone in chicken tissues are not shown to be adequate, even if nihydrazone could be shown to be an adequate marker for total residues. In the second study the method of analysis would not be sufficiently sensitive, based on the known tumorigenic properties of the drug. In the third study the method used, even if it could be shown that nihydrazone measurement at 2 ppb was adequately sensitive, is not aceptable because the supporting data on the method are not adequate to assure the reliability of results. Likewise, in the first study the results are so variable that a withdrawal period for the depletion of parent drug, nihydrazone, if nihydrazone were accepted as a marker at 2 ppb, could not be established with reasonable certainty, based on the numbers of animals used in view of data presented. The requirements of step 6 and section 512 (b) (8) and (d) (2) (D) of the act have not been fulfilled.

Therefore, the Director is hereby withdrawing the notice of opportunity for hearing issued March 31, 1971, proposing to withdraw approval of NADA's providing for the use of nihydrazone (NF–64) in food-producing animals. Under section 512(e) (1) (B) of the act (21 U.S.C. 360b(e) (1) (B), the Director proposes to withdraw approval of certain existing NADA's providing for the use of nihydrazone in food-producing animals on the grounds that such use violates section 512 of the act in that such use has not been shown to be safe.

This notice applies to all outstanding NADA's for the use of nihydrazone in food-producing animals. All such applications known to FDA are listed below by NADA number and by each applicant's name and last known address in FDA records. Copies of this notice are being forwarded by registered or certified mail to each of the known applicants. Notice to other applicants or holders of NADA's whose identity is not known to FDA is given by this publication.

13–195, Hess and Clark, Division of Rhodia, Inc., 7th and Orange St., Ashland, OH 44805.

13–200, Norwich Pharmacal Co., Division of Morton-Norwich Products, Inc., 13–27 Eaton Ave., Norwich, NY 13815.

Any holder of an approved application that elects to avail itself of an opportunity for hearing pursuant to section 512(e) (1) of the act and § 514.200 of Chapter I of Title 21 of the Code of Federal Regulations must file with the Hearing Clerk, Food and Drug Administration, Rm. 4–65, 5600 Fishers Lane, Rockville, MD 20852, a written appearance requesting such hearing by September 16, 1976, giving the reasons why approval of the application should not be withdrawn, and providing a well-organized and full-factual analysis of the scientific and other investigational data such holder is prepared to prove in support of its opposition to the Director's proposal. A request for hearing may not rest upon mere allegations or denials but must set forth specific facts showing that there is one or more genuine and substantial issue(s) of fact requiring a hearing. Responses to this notice and copies of published literature cited in this notice may be seen in the office of the Hearing Clerk, Food and Drug Administration, during regular business hours, Monday through Friday.

If a hearing is requested and is justified by any holder's response to this notice of opportunity for hearing, the issues will be defined, an Administrative Law Judge will be assigned, and a written notice of the time and place at which the hearing will commence will be issued as soon as practicable.

Any hearing on the withdrawal of approval of the foregoing NADA's will be open to the public. If, however, the Director finds that portions of the applications that serve as a basis for such a hearing contain information concerning a method or process that is entitled to protection as a trade secret, that part of the hearing will not be public, unless the respondent so specifies.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (sec. 512, 82 Stat. 343–361 (21 U.S.C. 360b)) and under authority delegated to the Commissioner (21 CFR 5.1) and redelegated to the Director of the Bureau of Veterinary Medicine (21 CFR 5.29) (recodification published in the FEDERAL REGISTER of June 15, 1976 (41 FR 24262)).

A discussion of the economic and environmental impact of this action is set forth in the notice of proposed rulemaking on nihydrazone, which is published elsewhere in this issue of the FEDERAL REGISTER.

It is hereby certified that the economic and inflation effects of this proposal have been carefully evaluated in accordance with Executive Order 11821.

Dated: August 6, 1976.

C. D. VAN HOUWELING,
Director, Bureau of Veterinary Medicine.

[FR Doc.76–23618 Filed 8–10–76;11:19 am]